Spawning, Larval Development and Recruitment of Scleractinian Corals in Tung Ping Chau Marine Park, Hong Kong

CHUI, Pui Yi

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Thesis Committee

Professor LM CHU (Chair)
Professor Put ANG, Jr (Thesis Supervisor)
Professor KM CHAN (Committee Member)
Professor LM CHOU (External Examiner)

Abstract

Coral recruitment is generally recognized as one of the key processes for the maintenance of coral communities. It is a crucial process involved in facilitating reef recovery following disturbance. Long term monitoring on the number of settlement and post-settlement survival of coral recruits may provide the baseline information for early warning of potential damage to coral community or impacts on its recovery after disturbance. In Hong Kong, previous researches have focused on the study of current state of the coral communities. There has been no detailed analysis on coral settlement and survival of early recruitment stages. Thus, this research is intended to fill in this knowledge gap on the recruitment states of Hong Kong corals and to provide an understanding on how coral spawning, interaction with other organisms and different environmental parameters can be linked with the patterns of coral recruitment.

Synchronous multi-specific spawning of scleractinian corals in Hong Kong was observed in May 2009, June and early July 2010, while synchronous spawning of only one scleractinian coral species was observed in late July 2010. Coral spawning was mostly observed from 19:30 to 22:00 hrs. A total of 12 species from eight genera and three families of scleractinian corals were observed to release gametes from

2009 to 2010. These 12 out of a total of 84 known coral species found in Hong Kong represented some of the most common and dominant ones. The majority of the species observed to spawn were from the family Faviidae. Eleven species were hermaphroditic, and one species was gonochoric.

The present study was successful in collecting egg-sperm bundles from the target species *Platygyra acuta*, and in achieving a high fertilization success (95.04%) of the eggs and sperms that were subsequently obtained. More than 200,000 larvae were reared, and induced to settle on artificial substrata that ended with 8,446 settled coral spats. Baseline information was obtained with respect to the embryonic development, larval rearing and competence of *P. acuta*, the most dominant coral in Hong Kong.

The overall data from settlement tiles and concrete blocks monitoring revealed the extremely low natural recruitment success of corals in Tung Ping Chau Marine Park (TPCMP), Hong Kong, with settlement tiles having a recruitment rate of 0.94 recruits/m^2 and that for concrete blocks, 16.61 recruits /m^2 throughout the 1.5 year study period. Although recruitment rate on concrete blocks appears to be higher, this was contributed mainly (90.9%) by the recruits of *Oulastrea crispata*, a pioneering species that easily colonizes newly opened space. This species has an encrusting

growth form. It thus cannot contribute to enhancing the 3-dimensional topographic complexity of the coral community and is not considered to be a significant reef builder because of its small colony size (< 5 x 5 cm^2). The overall low natural recruitment success was mainly due to low settlement rate of the coral larvae, which may have resulted from a lack of competent larvae retained within the sites, or the sources of larvae that regularly come to TPCMP may have been disturbed.

Recruits of *P. acuta* pre-seeded on ceramic tiles that were subsequently grown *in situ* showed that these coral recruits experienced very high mortality following settlement. Average mortality of 78.36% was observed within half a month of tile deployment *in situ*, and < 1% survived through the first four months of deployment. Low post-settlement survival was suggested to be a result of high sedimentation, intense competition for space with other fouling organisms and predation effects.

Predator exclusion experiments were carried out to examine the role of coral predators like corallivorous gastropods on coral recruit mortality. The results indicated that reducing predation effect may indirectly increase the growth of fouling organisms like oysters as they were also released from predation pressure. Thus, no increase in the survival rates of the coral recruits was detected under the "predator

free" condition. These results indicate a far more complex interaction among different organisms that contributed to coral recruitment failure. No single factor could be pinpointed as the key that leads to low settlement rate of coral larvae or high post-settlement mortality, hence the low recruitment success. Nonetheless, the intensity of fouling does provide some clues to the extent of competition with fouling organisms for space the coral recruits must face and how this could likely contribute to high post-settlement mortality of corals.

摘要

珊瑚幼新添入量通常被認爲是維持珊瑚群落的其中一個重要關鍵，是作爲協助珊瑚礁被干擾後恢復的一個關鍵性過程。透過長期監測珊瑚附著的數量和附著後新珊瑚附苗的生存率，可作爲珊瑚群落潛在損害的早期預警，或爲對受干擾後影響珊瑚群落恢復作基礎資料。在香港，以往的研究主要集中在調查珊瑚群落的現狀。目前還沒有詳細對珊瑚附著和附著後珊瑚附苗早期階段生存率的分析。正因如此，本研究課題的目的就是爲了補充本港珊瑚幼新添入量狀況方面缺少的資料，並探討有關珊瑚產卵，生物體之間的相互作用，及不同環境參數對於珊瑚幼新添入量的關係。

本研究於二零零九年五月，二零一零年六月及七月初觀察得多種石珊瑚品種進行同步產卵情況。而致二零一零年七月下旬只錄得一種石珊瑚品種的同步產卵情況。所觀察的石珊瑚產卵主要從晚上七時到十時進行。由二零零九至二零一零年，所觀察產卵的石珊瑚合共十二種，來自三科，八屬。然而這十二個石珊瑚品種卻代表了本港八十四種已知珊瑚品種中，一些最常見的和主導的品種。主要錄得產卵的石珊瑚品種是屬於蜂巢珊瑚科。其中雌雄同體的品種佔七個，只有一個是雌雄異體的品種。

本研究成功地從目標品種尖邊扁腦珊瑚中收集到配子束，及後成功取得 95%受精的卵子，飼養超過二十萬隻珊瑚幼虫，並成功誘導幼虫附著在人工基質上，獲得 8446 個珊瑚苗。本試驗爲蜂巢珊瑚科 (尖邊扁腦珊瑚 *Platygyra acuta*) ，在珊瑚胚胎發育，幼蟲飼養和反應能力的資料上作出貢獻。

香港東平洲海岸公園海域安置的附著板及混凝土塊的總體數據顯示了極低的成功天然珊瑚幼新添入量。在一年半的調查其間，平均附著板的珊瑚幼新添入量是 0.94 新增個體/平方米，而混凝土塊的珊瑚幼新添入量是 16.61 新增個體/平方米。雖然混凝土塊的珊瑚幼新添入量似乎較高，但主要添入種類以黑星珊瑚 (*Oulastrea crispata*)，珊瑚中的先驅品種爲主(90.9%), 它能優先地新添在新基質上。這種珊瑚只有表覆形的生長形態，群體通常比較細小扁平 (<5 x 5 平方厘米) ，不能有助於增強珊瑚群落複雜的立體地形，所以不被認爲是一個重要的珊瑚礁建設者。整體低珊瑚幼新添入量主要是由於幼生附著量偏低，可能是由於東平洲海岸公園海域缺乏有附著能力的幼蟲保留在內，或珊瑚幼蟲的來源地可能已被破壞。

預先誘導附著在陶瓷地磚上，再放到東平洲海岸公園海域的最常見珊瑚尖邊扁腦珊瑚(*Platygyra acuta*) 附苗的死亡率非常高。在首半個月內平均死亡率爲

78.36%，<1%能存活到四個月，低幼珊瑚附苗生存率有機會是受到沉積物的影響，以及與其它生物體之間激烈的空間競爭及被捕食影響而死亡。

進行排斥捕食者實驗是爲了研究珊瑚天敵腹足動物對附苗生存率的影響。研究結果顯示排斥捕食者有機會間接地增加"污損生物"的生長，例子如牡蠣，因爲它們也在捕食壓力中釋放。因此，在"沒有捕食者"的狀態下，並沒有增加幼珊瑚附苗的存活率。研究結果揭示了一個更爲複雜的不同生物體之間的相互作用，造成幼珊瑚新添失敗。沒有任何單一因素能劃分爲導致偏低的珊瑚幼蟲附著率或構成高幼珊瑚附苗的死亡率的重點，構成偏低的幼珊瑚新添入量。 儘管如此，嚴重的"污損生物"生長確實能提供一些線索，與"污損生物"之間激烈程度的空間競爭是幼珊瑚附苗必須面對的，而這很有可能是導致珊瑚附著後高死亡率的其中一個重要原因。

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Chapter 1

General Introduction and Thesis Outline

1.1 Introduction

1.1.1 Coral reefs and conservation

Coral reefs, often characterized as underwater tropical rainforest, are one of the most important marine habitats with high biodiversity and productivity (Hoegh-Guldberg, 1999). They also support the livelihood of millions of people (Moberg and Folke, 1999). Corals are slow growing animals, very delicate and sensitive to changes in environmental conditions. Around the world, coral reefs are in a state of decline because of multiple natural and anthropogenic stressors, including hurricanes (cyclones, typhoons), pollution, sedimentation, eutrophication, rising sea surface temperature and ocean acidification (Hoeghes and Connell, 1999; Hoegh-Guldberg, 1999; Hoegh *et al.*, 2007; Fabricius, 2011).

Knowledge of coral reproductive biology, subsequent dispersal and recruitment process are essential background information for the study of coral population and community dynamics as well as for facilitating reef restoration work (Harrison and Wallace, 1990). Knowing the timing of reproduction and the dispersal pattern can

help to track the sources of larvae and the dispersal route that the larvae may have followed, hence identifying the important coral communities to be protected. This could also help to understand the recovery processes after large-scale disturbance (Richmond, 1997; Hughes and Connell, 1999). Understanding the pattern of coral spawning is also important to allow gathering of coral gametes for a new range of studies investigating coral early life stages, including coral fertilization processes (Babcock, 1985; Oliver and Babcock, 1992; Willis *et al.*, 1997), embryogenesis (Babcock and Heyward, 1986; Nami and Motokawa, 2007), culture of larvae and their recruitment into the field for restoration work (Heyward *et al.*, 2002; Hatta *et al.*, 2004; Guest *et al.*, 2010), settlement, post-settlement development and survivorship of recruits under different environmental conditions (Babcock, 1985; Hodgson, 1990; Negri *et al.*, 2001; Kurihara, 2008; Suwa *et al.*, 2010).

Coral recruitment is generally recognized as one of the key processes for the maintenance of coral communities. As recruits are often more susceptible than adults to environmental changes (Babcock, 1985), the future state of a reef may better be evaluated by processes related to recruitment than by those related to the current state of the adults (Richmond, 1997). Long term monitoring on the number of settlement and post-settlement survival of coral recruits may provide the baseline information

for early warning of potential damage to coral community or impacts on its recovery after disturbance, and help in evaluating the effectiveness of management of a marine protected area (Richmond, 1997; Hughes and Connell, 1999). This understanding will definitely help towards future coral studies as well as effective management of marine ecosystem for the conservation of coral reefs and coral communities.

1.1.2 Reproduction in scleractinian corals

Corals exhibit both sexual and asexual reproduction (Figure 1.1). Sexual reproduction involves gametogenesis, spawning, fertilization of gametes, subsequent planktonic larval development, attachment to substratum, and metamorphosis to develop into a primary polyp. Asexual reproduction may occur through fragmentation or asexual production of planula larvae. It is important for the formation of adult colonies from a single polyp via asexual budding (Fadlallah, 1983; Harrison and Wallace, 1990; Richmond and Hunter, 1990; Harrison, 2011).

1.1.2.1 Sexual reproductive pattern

Corals show two aspects of sexual reproductive patterns. They can be hermaphroditic or gonochoric in their sexuality; and they can broadcast their gametes in mass/multi-specific spawning (broadcaster) or brood their larvae (brooder) as a mode

of larval development. Hermaphroditic corals have both male and female gonads developed within polyp or in different polyps within the same colony, whereas gonochoric corals have separate sexes at the colony level. Broadcast spawners carry out external fertilization in the water column, whereas brooders carry out internal fertilization within the maternal polyp and release planula larvae. Overall, based on the information on sexuality and mode of development for 400 of at least 900 extant hermatypic scleractinian species in the world, scleractinian corals can mostly be divided into four groups, including a) hermaphroditic broadcasters, the most dominant group (64.5%); followed by b) gonochoric broadcasters, the moderately common group (19.5%); c) hermaphroditic brooders and d) gonochoric brooders, relatively uncommon groups (Fadlallah, 1983; Harrison and Wallace, 1990; Richmond and Hunter, 1990; Harrison, 2011). However, there are still coral species which cannot be strictly categorized into one of the four main patterns of sexual reproduction. Some of these have mixed or contrasting patterns of sexuality or with both modes of sexual patterns exhibited. Some species are reported to brood asexually derived planulae (Harrison and Wallace, 1990; Harrison, 2011).

Broadcast spawning corals usually undergo one or two gametogenic cycles, while most brooding species have multiple gametogenic cycle each year (Szmant, 1986;

Harrison and Wallace, 1990). The breeding season for broadcast spawning species is relatively short and discrete. Spawning usually occurs synchronously throughout the population, concentrating in one or a few nights each year. Brooding species tend to have a relatively long breeding period; spawning of planulae is less synchronized and could occur over extended periods year-round, or may last over a few weeks (Harrison and Wallace, 1990). The number of research on sexual reproductive pattern of scleractinian corals has greatly increased since early 1980s and covered different geographical regions. This topic has been reviewed extensively by Harrison and Wallace (1990), Richmond and Hunter (1990), Richmond (1997); Guest *et al.* (2005) and Harrison (2011).

1.1.2.2 Overview of coral spawning patterns

Mass spawning is defined as the highly synchronous release of gametes by many individual coral colonies and species in one evening between dusk and midnight (Willis *et al.*, 1985), whereas multi-specific spawning is currently formally defined as the synchronous release of gametes by two or more species (Harrison, 2011). Spawning is usually synchronized within a population, but instead of spawning on a single night, split-spawning over consecutive nights or lunar months is relatively common in many populations (Willis *et al.* 1985; Babcock *et al.*, 1986, Harrison and

Wallace, 1990, Guest *et al.*, 2005). The causal factors for this split spawning phenomenon are still unclear. It is suggested to be a reproductive strategy to minimize the risk of catastrophic event (Richmond and Hunter, 1990), or to provide a second chance for not fully reproductive or stressed colonies (Van Veghel, 1994).

During mass/multi-specific spawning event, broadcast spawning species release gametes as compressed egg-sperm bundles or separately as eggs and sperm cloud from individual colony into water column for external fertilization. The bundles are positively buoyant due to their high lipid content and will rise up to the sea surface after being released from the polyps. Eggs and sperms then get dissociated by waves and all gametes from different colonies meet on the water surface for fertilization to occur, followed by subsequent embryonic and larval development. It usually takes 2.5-6 days for embryos to become fully developed larvae. Competent larvae are then able to settle and metamorphose into single polyps. Newly settled polyps will subsequently develop and deposit calcium carbonate skeleton (Babcock and Heyward, 1986; Harrison and Wallace, 1990; Miller and Mundy, 2003)

Mass spawning was first documented in the Great Barrier Reef (GBR), Australia in the early 1980s (Harrison *et al.*, 1984). A total of 105 coral species, from 36 genera,

11 families were observed to spawn over seven nights, representing approximately one-third of the 340 species found on the GBR. More than 20 species of corals were recorded to spawn on the same night (Babcock *et al.*, 1986). Since then, lots of studies have been done on coral reproductive pattern in different regions around the world, including Australia (Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986; 1994; Wilson and Harrison, 2003), the Red Sea (Shlesinger and Loya, 1985; Shlesinger *et al.*, 1998), the Caribbean (Szmant, 1986), Guam in the western Pacific (Richmond and Hunter, 1990; Richmond, 1997), Japan (Heyward *et al.*, 1987; Hayashibara *et al.*, 1993), Taiwan (Dai *et al.*, 1992), the Philippines (Vicentuan *et al.*, 2008), Singapore (Guest *et al.*, 2002; Guest *et al.*, 2005), Thailand (Kongjandtre *et al.*, 2010) and Kenya (Mangubhai and Harrison, 2008) (see also review by Harrison, 2011). However, despite being the region with the highest diversity of scleractinian corals in the world, very limited information is known about coral reproduction in Southeast Asia, including the South China Sea region (Guest *et al.*, 2005; Kongjandtre *et al.*, 2010).

1.1.2.3 Environmental factors regulating coral reproduction

The reasons and mechanisms for mass or multi-specific spawning are not clearly known, but it is believed to be an adaptive strategy. Since corals are sessile

organisms incapable to aggregating for reproduction, synchronous release of gametes for external fertilization is crucial for reproductive success. High degree of synchronized spawning maximizes fertilization rates by increasing the chance for gametes from conspecific individuals to meet in the water column/surface. It has been suggested that coral spawning is synchronized by taking annual sea temperature change as a seasonal cue; lunar phase as a fine tuner for nights; and onset of darkness and tidal regimes as ultimate cues to determine the actual timing of spawning (Shlesinger and Loya, 1985; Babcock, 1986; Harrison and Wallace, 1990; Richmond and Hunter, 1990, Richmond, 1997). Furthermore, it is suggested that the predictable precise spawning time may be related to the need to increase the rate of fertilization success by preventing gametes from drifting away by current before fertilization takes place, by reducing sperm dilution effect and by avoiding predation (Babcock *et al.*, 1986; Richmond and Hunter, 1990; Oliver and Babcock, 1992; Willis *et al.*, 1997). However, the reasons for multi-specific synchronous spawning, which may lead to gamete wastage due to between species hybridization or difficulty in finding and recognizing homospecific gametes, are less clear. Synchronous spawning may be the result of species responding similarly but independently to physical factors that maximize reproductive success during spawning (Harrison *et al.*, 1984; Babcock *et al.*, 1986; Guest, *et al.*, 2005).

1.1.3 Coral recruitment

Coral recruitment is generally recognized as one of the key processes for the maintenance of coral communities and for facilitating recovery after disturbance (Johnson and Preece, 1992; Connell *et al.*, 1997; Richmond, 1997). In general, recruitment success is affected by certain pre-settlement and post-settlement conditions, including the availability of competent larvae in the site (which is then related to gamete production, fertilization and the length of planktonic larval stage), the probability that the larvae will settle, and the probability that new recruits will survive after settlement (Keough and Downes, 1982; Connell *et al.*, 1997; Abelson *et al.*, 2005).

Following successful spawning, fertilization and subsequent embryonic development, planula larvae from broadcasting corals require minimum planktonic development period of 2.5 to 6 days to become competent to settle (Babcock and Heyward, 1986; Harrison and Wallace, 1990; Miller and Mundy, 2003). Although maximum competency can be up to several weeks, maximum settlement rates of coral larvae usually lie between 10 to 32 days after spawning (Wilson and Harrison, 1998). For brooding corals, planula larvae are able to settle immediately. Maximum settlement occurs within a few days after planulae release, but planula larvae can also survive

and settle up to 100 days (Richmond, 1987; Harrison and Wallace, 1990).

Dispersal ability of larvae varies between brooding and broadcasting corals. Because of the planktonic developmental period of larvae from broadcasting coral, the larvae of brooding corals are generally considered to have lower dispersal ability than those of the broadcasting ones. Self-seeding is more likely to occur in brooding than in broadcasting corals (Harrison and Wallace, 1990). To date, the relationship between dispersal ability of larvae and whether the reef is self-seeding or depends on larvae from other source reefs remains a strongly debated topic (Harrison and Wallace, 1990).

Settlement is a process by which a competent coral larva attaches itself to the substratum and metamorphoses into a juvenile polyp 'spat', while recruitment refers to the point at which new juvenile coral 'recruit' becomes visible to census (Keough and Downess, 1982; Connell, 1985; Harrison and Wallace, 1990). Because of the cryptic settlement behavior, small size (often $\leqq$2 millimeter in diameter) and slow growth rate of newly settled corals (Babcock, 1985; Babcock *et al.*, 2003), it is difficult or often impossible to detect them by naked eye. It may take up to a year before a coral recruit can become easily observable *in situ* (Wallace and Bull, 1981).

During this early stage of post-settlement, mortality is typically high due to local physical conditions or interaction with other organisms, e.g. competition (Tomascik, 1991; Hunte and Wittenberg, 1992; Lirman, 2001; McCook *et al.*, 2001; Abelson *et al.*, 2005; Fabricius, 2011; Chadwick and Morrow, 2011), predation (Turner, 1994; Morton and Blackmore, 2009), sedimentation stress (Sammarco, 1980; Rogers *et al.*, 1984; 1991; Hodgson, 1990; Tomascik, 1991; Fabricius, 2005; 2011) and different environmental disturbances (Babcock, 1985; Richmond and Hunter, 1990; Wilson and Harrison, 2005).

Since 1980s, *ex situ* microscopic examination of removable artificial settlement tiles has been used widely to study coral settlement and recruitment patterns (Harrison and Wallace 1990). Recently, florescence census technique developed can also help by making coral recruits much easier to observe *in situ* than in the current census methods under white light (Piniak *et al.,* 2005; Roth and Knowlton, 2009). Using this census technique, up to 97.6% of all coral recruits bigger than one millimeter in diameter can be detected *in situ* (Schmidt *et al.*, 2008). This technique greatly helps in rapid estimation of the location and abundance of any new recruits *in situ*, allows continuous *in situ* monitoring of recruits on artificial settlement tiles, more accurate determination of the settlement and early post-settlement survival of corals, and

assists the estimation of early growth of coral recruits (Baird *et al.*, 2006).

1.2 Coral Communities in Hong Kong

Hong Kong is located at the northern part of the South China Sea, a subregion in the Indo-West Pacific that is characterized with high coral diversity. Colder winter seawater temperature that drops to 14-16°C creates a marginal environment for reef building scleractinian corals. Under the influence of the freshwater discharged from Pearl River to the west, large region of Hong Kong water, especially the western part, is relatively turbid and low in salinity, hence, is not suitable for coral growth. Therefore, coral communities are mainly concentrated in the eastern and northeastern area of Hong Kong where a true oceanic condition exists and the influence of the Pearl River runoff is least.

Hong Kong has a relatively high diversity of 84 scleractinian coral species (Ang *et al.*, 2003). Previous studies have focused on the state of the coral communities and management plans (reviewed by Ang *et al.*, 2006; Tam and Ang, 2008) with relatively few reports published on the reproductive patterns of scleractinian corals, coral spawning and recruitment (Lam, 2000; Lin, 2003). None of these studies shows detailed monitoring of *in situ* coral spawning pattern and detailed analysis on coral

settlement and post-settlement survival of early recruitment stages. It is important to have a good understanding of coral spawning pattern and recruitment process to help track the sources of larvae and their dispersal route in order to identify the important coral communities that should be protected. This will also allow early warning to be made of any potential damage to these coral communities and evaluation of impacts on their resilience after disturbance. This understanding will form the basis for future coral studies, design of effective management strategies for the conservation as well as restoration of coral communities.

1.3 Objectives

This thesis research project, therefore, aimed at

1. Monitoring and examining coral spawning pattern, duration and timing of spawning, and the associated fertilization success with a detailed examination of the early developmental biology of *Platygyra acuta*, the most dominant coral species in Hong Kong.
2. Quantifying the settlement and post-settlement survival of coral recruits at multiple spatial (site, depth) and temporal (season) scales on artificial substrata using both microscopic and fluorescence census techniques
3. Examining the relationships between the rate of larval settlement and the timing

of coral spawning in the study sites.

1.4 Study Sites - Tung Ping Chau Marine Park

Tung Ping Chau Marine Park (TPCMP) (22°32 N, 114°25 E) is an island located in the north-eastern side of New Territories, Hong Kong Special Administrative Region (HKSAR), China (Figure 1.2). TPCMP was designated as the fourth marine park in Hong Kong in November, 2001 and part of the National Geopark in November, 2009. It is regarded as an important resource for marine conservation and an ideal site for conducting scientific research. It is relatively remote from the rest of Hong Kong; the public can only visit the island during public holidays and weekends due to limited transportation access. Therefore, the water around TPCMP is relatively clean and free from human pollution impacts.

The two coral core areas located along the eastern side of TPCMP, A Ye Wan (AYW) and A Ma Wan (AMW), where coral diversity was one of the richest in Hong Kong, were selected as the sites for this study. These sites support high coral coverage of over 60%, with 45 of the 84 scleractinian coral species recorded from Hong Kong (Ang *et al.*, 2003). Corals are mostly abundant in depths that ranged from -1 m CD (Chart Datum) to -4 m CD. Among the two sites, AMW has a richer coral diversity

(Ang *et al.*, 2000). Zonation pattern of coral communities was observed in both sites, with shallow water zone, 0 to -1 m CD, having significantly different coral communities than deeper water zone deeper than -1 m CD. Substratum for both sites, from 0 to -1 m CD, is mainly composed of rocks; whereas from -1 m to -3 m CD, it is mainly sandy to silty (Tam and Ang, 2007).

1.5 Thesis Outline

This thesis is divided into four chapters and a brief description of each chapter is given below:

Chapter 1 – General Introduction and Thesis Outline

This chapter provides a general introduction to coral reproduction and recruitment, focusing on researches that have been done on these topics. A description of the study sites and the objectives of the study are also given.

Chapter 2 – Spawning of Scleractinian Corals in Hong Kong, Larval Culture and Development

This chapter describes the timing of spawning and attempts to relate these to environmental variables. It also describes the results of experiments on fertilization

success, culture of coral larvae, embryonic and early developmental biology of *Platygyra acuta*, the most dominant coral species in Hong Kong, as well as induced settlement of *P. acuta* larvae on artificial substrata.

Chapter 3 – Recruitment Patterns of Scleractinian corals in Tung Ping Chau Marine Park, Hong Kong

This chapter examines the *in situ* larval settlement pattern on terracotta tiles provided as artificial substrata and relates it to coral spawning patterns documented in Chapter 2. It also describes the results of experiments carried out to evaluate the *in situ* post-settlement survivorship of the corals and suggests possible factors that cause variations in the rates of recruitment and recruit mortality. The implications of these variations on community resiliency are discussed.

Chapter 4 – Summary and Perspectives

This chapter summarizes the major findings of experiments carried out in this thesis research. The significance of these findings is discussed with suggestions given for future directions of study. These findings provide the essential background information for further understanding of coral population and community dynamics. How these could help to facilitate restoration work on disturbed coral communities,

as well as the design of effective management strategies for the conservation of coral communities in Hong Kong are examined.

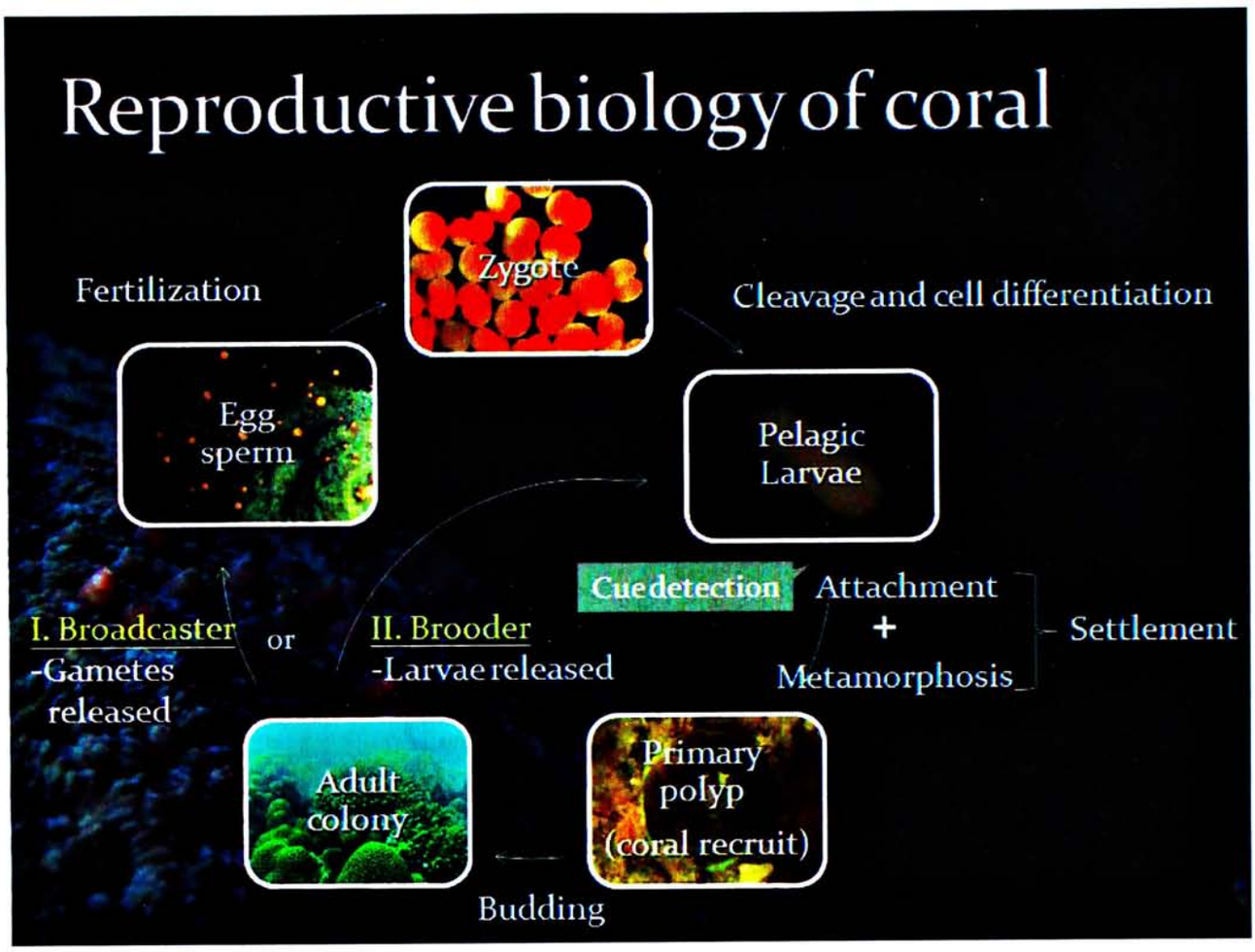


Figure 1.1 Schematic flow chart showing processes involved in the reproductive biology of coral.

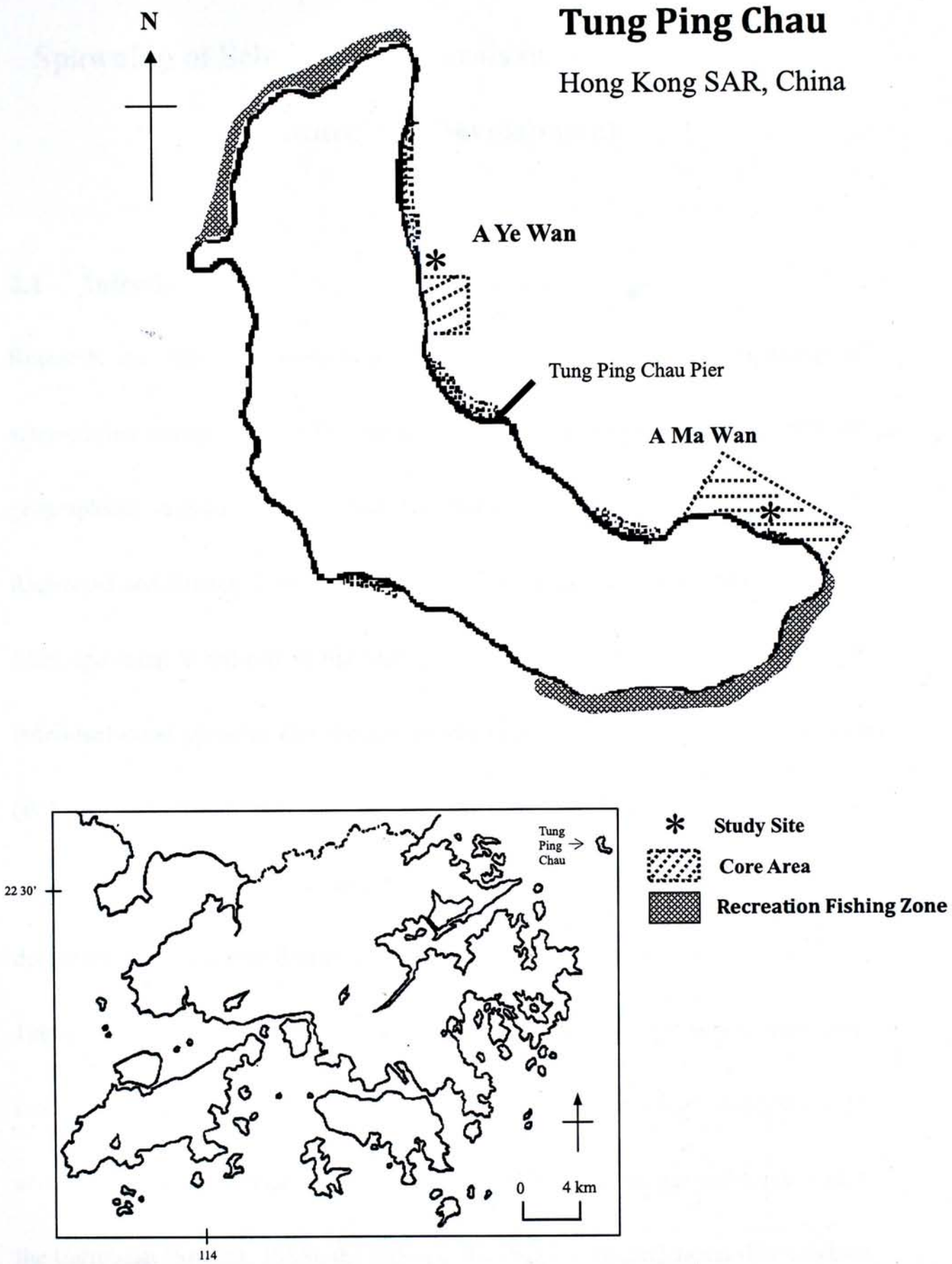


Figure 1.2 Map of Hong Kong and Tung Ping Chau Marine Park showing the location of the study sites A Ye Wan (AYW) and A Ma Wan (AMW).

Chapter 2

Spawning of Scleractinian Corals in Hong Kong, Larval Culture and Development

2.1 Introduction

Research on sexual reproduction and mass or multi-specific spawning of scleractinian corals has greatly increased in number and expanded to different geographical regions over the last two decades (Harrison and Wallace, 1990; Richmond and Hunter, 1990; Richmond, 1997; Guest *et al.*, 2005; Harrison, 2011). Mass spawning is defined as the highly synchronous release of gametes by many individual coral colonies and species in one evening between dusk and midnight (Willis *et al.*, 1985), while multi-specific spawning refers to the synchronous release of gametes by two or more species (Harrison, 2011). Mass spawning was first documented in the Great Barrier Reef, Australia in the early 1980s (Harrison *et al.*, 1984). Since then, many other studies have been carried out to examine coral reproductive patterns in different regions around the world, such as Australia (Willis *et al.*, 1985; Babcock *et al.*, 1986; 1994), the Red Sea (Shlesinger and Loya, 1985), the Caribbean (Szmant, 1986), the Indo-Pacific region including Japan (Hayashibara *et al.*, 1993), Taiwan (Dai *et al.*, 1992), the Philippines (Vicentuan *et al.*, 2008),

Singapore (Guest *et al.*, 2002; Guest *et al.*, 2005), and Thailand (Kongjandtre *et al.*, 2010) (see review by Harrison 2011).

However, still very limited information is known about the timing of coral reproduction in Southeast Asia, a region with the highest diversity of scleractinian coral species in the world (Guest *et al.*, 2005; Kongjandtre *et al.*, 2010). Hong Kong, located at the northern limit of Southeast Asia region, with high coral diversity of 84 scleractinian coral species recorded (Ang *et al.*, 2003), has only a few documentations (e.g. Lin, 2003) on the reproductive patterns of scleractinian corals and the timing of spawning.

Understanding the timing of coral spawning is important to help predict the fertilization success that may be affected by weather condition during spawning (e.g. storm and rainfall) and the dispersal of coral larvae. It also allows gathering of coral gametes to facilitate a new range of studies, including investigations on coral fertilization rates, embryogenesis, culture of larvae and seeding of recruits onto artificial substrata for use in the enhancement of recruitment success and for the restoration of degraded coral communities. Studies on the settlement processes, post-settlement development and survivorship of recruits could also be carried out.

All these will be essential for the development of effective management strategies of a marine protected area and the evaluation of effects of pollution or disturbances on coral population and community dynamics (Harrison and Wallace, 1990; Oliver and Babcock, 1992; Heyward *et al.*, 2002; Hatta *et al.* , 2004; Guest *et al.*, 2010).

Therefore, the aims of this experiment were:

1. To document the pattern, duration and timing of scleractinian coral spawning in Hong Kong, particularly in Tung Ping Chau Marine Park (TPCMP);
2. To follow the embryonic development of *Platygyra acuta* larvae, the most dominant coral species in Hong Kong;
3. To apply the protocol for the culture and seeding of coral larvae (*Platygyra acuta*) on artificial settlement plate for use in coral restoration work.

2.2 Methods and Materials

2.2.1 Site description

Tung Ping Chau Marine Park (TPCMP) (22°32 N, 114°25 E) is an island located in the north-eastern side of New Territories, Hong Kong Special Administrative Region, China (refer to Chapter 1 for details). A Ye Wan (AYW), one of the two main patches of coral communities on the eastern side of the island, was selected for the

spawning observation. Corals in this study site are mainly concentrated in the water depth of -0.5 to -2m below Chart Datum (CD).

2.2.2 Spawning observation

Nighttime observations were carried out *in situ* in AYW, TPCMP for two years (2009 and 2010). Observation periods were of different durations that ranged from one to eight consecutive nights due to logistic or weather constraints. Four spawning checks were conducted in total, after full moon in May 2009, May, June and July 2010, every night from 19:00 to 22:30 hrs along the coast, with water depth ranging from -0.5 to -2 m CD. Any coral colony encountered spawning or being ready to spawn (setting) was identified and photo recorded. Spawning checks in May 2009 and May 2010 were targeted for collection of egg-sperm bundles of *Platygyra acuta*, the most dominant coral species in the study site. Therefore, more extensive monitoring of coral spawning was made only in June and July 2010. No spawning check in August 2010 could be carried out due to typhoon.

2.2.3 Target coral species, *Platygyra acuta*

Platygyra acuta belongs to the family Faviidae with massive growth form that can be over 1 m in height. The corallites are typically sub-meandroid or monocentric.

They have slightly exert, ragged septa and acute walls. The colony is usually dark-light brown in color. This species is commonly found in shallow waters. It is a hermaphroditic broadcast spawner. A more detailed spawning behavior of this species was monitored in the present study.

2.2.4 Coral gamete collection

In the spawning checks in 2009 and 2010, bundle collectors (Figure 2.1) were used to collect egg-sperm bundles released from *Platygyra acuta* after full moon in May 2009 and May 2010. May 2009 was a trial run, with modifications on the technique done in May 2010. Ten individual colonies of *P. acuta* were tagged in the field in AYW, TPCMP at depths of -0.5 to -1.5 m CD in May 2010. Previous monitoring studies carried out in our laboratory (Ang *et al.*, 2010) showed that spawning would normally occur not earlier than the fourth day after full moon. Corals were therefore examined every night starting from the third night after full moon. A 450 μm-bundle collector was pre-set on top of each of the five tagged coral colonies just before sunset (around 18:30 hr). The collector was fastened by strings and carefully weighed down on four sides without touching the coral. If no spawning occurred, the bundle collector would be removed and reset in the following night until spawning occurred. For the other five tagged colonies, spawning was closely

monitored by divers equipped with a 150 μm-bundle collector every night from 19:00 to 22:30 hrs. Once the coral colony started to show signs of ready to spawn, usually characterized by bloated oral discs with emerging egg-sperm bundles (the setting stage), then a collector would be placed over the colony or part of the colony to collect the bundles as soon as when they were released from the coral polyps.

During spawning, eggs and sperms were packed and released as egg-sperm bundles from the polyps of *P. acuta*. This phenomenon is true also for most other Faviids observed. The bundles are positively buoyant so will slowly rise up to the sea surface after being released. The bundles would then be broken up by waves, dissociating the eggs from the sperms. With massive spawning from different colonies at the same time, all gametes from different colonies will then mix on the water surface where fertilization occurs. By setting the bundle collectors right over the coral colonies, bundles from a single colony can be trapped and collected in collecting bottles (Figure 2.2). The bottles were closed while underwater, then brought back immediately to the make-shift laboratory set up in TPCMP for further processing (Figure 2.3).

2.2.5 Fertilization and larval rearing

The procedures adopted in this part of the experiment followed those described in Hatta *et al.* (2004) and Guest *et al.* (2011). Egg-sperm bundles from different colonies collected in different bottles were mixed in a fertilization tank filled to a final volume of 20 L with 40 μm freshly filtered seawater. The water in the tank was gently stirred to break up the bundles, releasing eggs and sperms to allow fertilization to take place. All eggs floated on the surface formed a pink layer and the sperm caused the water to become milky in color. The concentration of sperm was determined using a haemocytometer.

One hour after mixing, all eggs on the surface were skimmed and transferred to another rearing container with 60 L 40 μm filtered seawater. This procedure was repeated again to allow sperm wash, as the remaining sperms would cause deterioration of the water quality after death. After the sperm wash, eggs were skimmed to several rearing containers and filled with fresh 40 μm filtered seawater to a final volume of 60 L, to keep the eggs less dense and to ensure better water quality.

Three hours after mixing, fertilization rate was calculated by taking three subsamples from one of the rearing containers. The number of fertilized eggs was counted under the dissecting microscope and the proportion calculated as a percentage of the total number of eggs in each subsample. Eggs which appeared in 2-cell or multiple-cell stages were considered as fertilized, while unfertilized eggs remained spherical in shape (Figure 2.4). The embryos in rearing containers were left undisturbed for 12 hrs under ambient temperature with no aeration. In the meanwhile, occasional gentle stirring was applied if the floating embryos were observed to clog together, especially along the margin of the tanks. Twelve hours after mixing, the developing embryos were skimmed again and transferred very carefully to a new rearing container with fresh 40 μm filtered seawater filled up to a final volume of 60 L. These steps were then repeated every 24 hrs. When the larvae started to swim, they were skimmed and transferred to new rearing containers each with a final volume of 20 L. Thereafter, 20 L fresh 40 μm filtered seawater were added every 24 hrs to the containers instead of skimming to transfer the larvae to new containers. Total larval counts were estimated by taking three 50 ml sub-samples from all of the rearing containers and counting the number of larvae in each sub-sample under the dissecting microscope.

2.2.6 Embryonic development of *Platygyra acuta* larvae

Egg-sperm bundles were observed under the dissecting microscope. Early stages of development were examined by sampling from fertilization or culture tank at hourly intervals during the first 12 hrs after fertilization (t=0 to t=12), every 2 hrs from t=12-24, every 4 hrs from t=24-72 and every 12 hrs thereafter. Approximately 50 eggs or embryos of *Platygyra acuta* were collected during each sampling and fixed in 2.5% Glutaraldehyde (GA) for 4 hours at 4℃. Fixed samples were washed by 0.2M phosphate buffer (PBS) after 4 hrs and post-fixed in 1% buffered osmium tetroxide at 4℃. Post-fixed samples were washed by 0.2M phosphate buffer (PBS) and kept in Eppendorf tubes until ready to be processed for Scanning Electron Microscopy (SEM) examination. To prepare the samples for SEM, the samples were dehydrated in increasing concentration of acetone-ethanol (50%, 70%, 80%, 85%, 90%, 95%, 95% and anhydrous acetone) baths, at 10 min in each bath. Drying was carried out by using anhydrous acetone as intermediate liquid and tertramethylsilane (TMS) as the transition liquid. After 15 min, tertramethylsilane was removed from the samples and the samples allowed to dry at 30℃ in air for 10 min, until evaporation of the organic compounds was complete.

After the drying stage, samples were mounted with the aid of an elongated pipette

tube on stubs with double sided conductive sticky tape. In this way, the eggs or embryos could be oriented in all possible directions. Finally, the eggs or embryos were observed under the SEM with a working voltage of 20 kV.

2.2.7 Induced settlement of coral larvae

Two types of ceramic tiles, 44 and 117 cm^2 in size, were used as artificial substrata for larval settlement. Tiles were pre-conditioned in A Ma Wan shallow water, on the eastern side of TPCMP, for 1.5 months to allow the development of biofilm and crustose coralline algae (CCA) on them. Preliminary studies have indicated that at least 1.5 months were needed for biofilm and CCA to develop on these tiles. When majority of the larvae showed competent behavior (i.e. swimming down and crawling over available bottom surfaces, which is thought to be actively selecting suitable substrata for attachment and metamorphosis (Fadlallah, 1983; Harrison and Wallace, 1990) around 6.5 days after spawning, tiles were collected back from the field. They were gently brushed to remove any biofouling organisms like tubeworms and barnacles which might inhibit larval settlement and affect the subsequent growth of newly settled larvae. These tiles were then arranged in pairs on rods, with each pair separated by a 5 cm plastic tube (Hatta *et al.*, 2004). The rods were hanged vertically in a settlement tank with 1000 L 40 μm filtered seawater to expose the

horizontal surface of the tiles (Figure 2.5). The tank was moderately aerated. All larvae from the rearing tanks were then collected by sieve and introduced to the settlement tank. After 48 hrs, the rods were turned upside down for larvae to settle on the other side of the tiles for another 24 hrs. Thereafter, the number of settled coral spat (settled larvae) on each tile was counted and their locations on the tile surface mapped under the dissecting microscope with the help of the fluorescence census technique (Baird *et al.*, 2006). These tiles with induced coral larval spats were used for post-settlement survival experiments described in Chapter 3.

2.2.8 Environmental cues that triggered the spawning events

Three main environmental cues were considered to be important in triggering coral mass spawning, including temperature, tidal level and lunar phases (Shlesinger and Loya, 1985; Babcock, 1986; Harrison and Wallace, 1990; Richmond and Hunter, 1990, Richmond, 1997). Minilog-T temperature loggers (Vemco, Halifax, Canada) were deployed from April 2009 to December 2010 in AYW, and from Jan 2010 to Dec 2010 in AMW to record the *in situ* temperature conditions at every 30 min interval. Information on tidal level and moon phases were obtained from the Hong Kong Observatory (Hong Kong Observatory 2009a, 2010a). Data on these environmental factors were matched with the actual spawning time of the corals to

find out if any pattern could be inferred to suggest a linkage between these factors and the spawning of corals.

2.3 Results

2.3.1 Spawning observation

Synchronous multi-specific spawning of scleractinian corals in Hong Kong was observed in May 2009, early June, and early July 2010, while synchronous spawning of colonies of only one species was observed in late July 2010. Coral spawning was mostly observed from 19:30 to 22:00 hrs. A total of 12 species from eight genera and three families of scleractinian corals were observed to release gametes in 2009 and 2010 (Table 2.1). The majority of these species belong to the family Faviidae. Eleven species were hermaphroditic, and one species was gonochoric. Egg-sperm bundles were observed to be released from hermaphroditic coral colonies, while eggs and clouds of sperm were observed to be released from separate colonies of gonochoric corals. Most species released their gametes as compact discrete bundles, red, pink to orange in color (Figure 2.6), while *Goniastrea aspera* bundles appeared to be loosely packed and grey in color (Figure 2.7). Most of the observed spawning behaviors belonged to Type I defined by Babcock *et al.* (1986), i.e. the slow release of egg-sperm bundles through the mouth of polyps throughout the colony in a period of

5 to 50 min. *Goniastrea aspera*, on the other hand, ejected egg-sperm bundles showing a Type II behaviour, i.e. rapid contraction over all or part of the colony after a short period of setting that was repeated once or several times. All egg-sperm bundles observed were positively buoyant. A more detailed account of the spawning checks in 2009 and 2010 is given below. Environmental conditions associated with each spawning event, namely the water temperature, day after full moon and tide levels, are also given.

2.3.1.1 May 2009

In May 2009, spawning checks started on 13 May, 4th night after full moon (9 May) and continued for five consecutive nights till 17 May. Coral spawning was first observed during the night of 16 May, the 7th day after full moon. Surface slicks (Figure 2.8) resulting from synchronous spawning event were observed starting at 19:30 hr. The water temperature was 25.9℃ (Figure 2.9A) and coral spawning occurred during an outgoing tide. The tide level reached the lowest of +0.4 m CD at 22:46 hr, and the highest tide of +1.55 m CD was recorded in the following morning at 05:55 hr. Spawning was observed for two consecutive nights and the species involved included *Favia rotumana*, *Platygyra acuta* and *P. carnosus*. The sea was calm during those two days.

2.3.1.2 May-Early June 2010

In 2010, more detailed spawning checks started on 31 May, 3rd night after full moon (28 May) and continued for eight consecutive nights till 7 June. Coral spawning was first observed during the night on 3 June, the 6th day after full moon. Spawning was observed for five consecutive nights. Observed spawning scleractinian corals included *Acropora tumida, Cyphastrea chalcidicum*, *Favia rotumana*, *Goniastrea aspera*, *Platygyra acuta* and *P. carnosus.*

On 3 June, no spawning colonies were observed till 20:00 hr. A number of *Favia rotumana* colonies were seen in setting stage (with tentacles retracted, oral disc distended, and egg-sperm bundles appearing below oral disc of polyps). At 20:30 hr, first spawning was observed and *F. rotumana* began to expel a large number of discrete, compact and positively buoyant egg-sperm bundles. The water temperature was 25.7℃ at 20:30 hr (Figures 2.9A, B) and coral spawning occurred in an incoming tide. The tide level was at the lowest of +0.7 m CD at 20:30 hr, and reached the next high of +1.3 m CD at 03:00 hr (Figure 2.10A).

On 4 June, spawning in several species began at 19:30 hr and lasted till 22:30 hr. Observed spawning scleractinian corals included *Acropora tumida, Favia rotumana*,

Platygyra acuta and *P. carnosus*. The spawning of *F. rotumana* was observed at 19:30 to 20:00 hr, followed by *A. tumida* at 20:30 hr. *P. acuta* and *P. carnosus* began setting (Figure 2.11) at 20:30 hr and spawned (Figure 2.12) from 21:00 to 22:30 hr. A large number of egg-sperm bundles was released from the colonies due to their massive growth form. The water temperature was 26.2℃ at 19:30 hr (Figure 2.9B) and spawning started in an outgoing tide, followed by an incoming tide. The tide level was +0.8 m CD at 19:30 hr, reached the lowest of +0.75 m CD at 20:30 hr, with the subsequent high tide of +1.45 m CD recorded at 05:00 hr in the following morning (Figure 2.10A).

On 5 June, spawning began at 20:00 and lasted till 22:00 hr. Observed spawning corals included *Cyphastrea chalcidicum*, *Favia rotumana*, *Platygyra acuta* and *P. carnosus*. The setting of *F. rotumana* was observed at 20:00 hr and spawning of one colony of *C. chalcidicum* was observed at 20:15 hr. Setting of *P. acuta* and *P. carnosus* began at 20:50 hr and multiple colonies spawned from 21:00 to 22:00 hrs. The water temperature was 26.2℃ at 20:00 hr (Figure 2.9B) and spawning occurred during a switch from outgoing to incoming tides. The tide level was +0.85 m CD at 20:00 hr and reached the lowest level of +0.8 m CD at 21:00 hr (Figure 2.10A). The highest tide was +1.55 m CD at 05:00 hr of the following morning.

On 6 June, coral spawning began at 21:00 hr and lasted for one hour. Observed spawning species included *Platygyra acuta* and *Goniastrea aspera*. Setting of *P. acuta* began at 20:50 hr and spawning was synchronous at around 21:00 hr. Some sporadic spawnings continued to be observed till 22:00 hr. Forceful expulsion of bundles via rapid contraction was observed in one *G. aspera* colony at 22:00 hr. All observed bundles from *P. acuta* were compact and red to orange in color, while *G. aspera* bundles appeared to be loosely bounded and grey in color. The water temperature was 26.2℃ at 21:00 hr (Figure 2.9B) and spawning occurred at an incoming tide. The tide level was lowest at 21:00 hr at +0.9 m CD and reached the highest of +1.65 m CD at 06:00 hr of the following morning (Figure 2.10A).

On 7 June, small scale spawning began from 21:00 till 22:00 hrs. Observed spawning species included *Goniastrea aspera* and *Platygyra acuta*. *G. aspera* was observed to spawn at 21:22 hr. Setting of *P. acuta* began at 20:50 hr and spawning occurred from 21:00 to 22:00 hrs. Spawning was not as vigorous as in the previous nights. One thing to be noted was the observation of a split spawning from a tagged *P. acuta* colony on 6 and 7 June. Patchy spawning of different parts of the same colony was observed around the same time at both nights. The water temperature

was 26.9℃ at 21:00 hr (Figure 2.9B) and coral spawning occurred in an outgoing tide. The tide level was +1 m CD at 21:00 hr and reached the lowest of +0.9 m CD at 22:00 hr (Figure 2.10A).

2.3.1.3 Late June 2010

In late June 2010, spawning checks started on 30 June, 4th night after full moon (26 June) and continued for six consecutive nights till 5 July. First sign of coral spawning was observed during the night of 2 July, the 6th day after full moon. Spawning was observed for three consecutive nights and no spawning was observed on the last day of the spawning check. Observed spawning scleractinian corals included *Acropora digitifera*, *A. tumida*, *Favites abdita*, *F. acuticollis*, *F. pentagona*, *Goniastrea aspera*, *Leptastrea purpurea*, *Platygyra acuta* and *P. carnosus*.

On 2 July, the first day of spawning, only one colony of *Goniastrea aspera* was observed to spawn at 21:56 hr. Loosely packed grey colored egg-sperm bundles were released from the colony. The water temperature was 29.6℃ at 22:00 hr (Figures 2.9A, C) and coral spawning occurred in an incoming tide. The tide level was +1 m CD at 21:30 hr (Figure 2.10B). The subsequent high tide occurred at 03:00 hr, reaching +1.4 m CD.

On 3 July, spawning began at 20:30 and lasted till 22:00 hr. Observed spawning coral species included *Favites abdita*, *F. acuticollis*, *F. pentagona*, *Goniastrea aspera* and *Platygyra acuta*. A number of *F. pentagona* colonies were observed to spawn from 20:30 to 21:00 hrs. In the meanwhile, *F. abdita*, *F. acuticollis* and *G. aspera* spawned at 20:45, 20:55 and 21:03 hrs respectively. The synchronous spawning of *P. acuta* was apparently missed. Only a layer of egg-sperm bundles on the water surface was observed, with very small amount of egg-sperm bundles remaining on the surface of the colonies, suggesting that *P. acuta* had just spawned at around 21:50-22:00 hrs. The water temperature was 30.04℃ at 20:30 hr (Figure 2.9C) and spawning occurred at the changing tide from the lowest tide level of +0.8 m CD at 20:30 hr to the incoming tide of the highest level of +1.5 m CD at 04:00 hr (Figure 2.10B).

On 4 July, spawning began from 21:00 till 22:00 hrs. Observed spawning species included *Acropora digitifera, A. tumida*; *Leptastrea purpurea*, *Platygyra acuta* and *P. carnosus*. Setting of *L. purpurea* was observed at 20:50 hr and spawning of *A. tumida* and *A. digitifera* was observed at 21:26 and 21:52 hrs respectively. For *Acropora* spp., only very small portion of the colonies was observed to spawn.

Setting of *P. acuta* and *P. carnosus* began at 20:50 hr and egg-sperm bundles were spawned at 21:30 to 22:00 hrs. The water temperature was 30.4℃ at 21:00 hr (Figure 2.9C) and spawning occurred at an incoming tide. The tide level was +0.9 m CD at 21:00 hr and reached the highest level of +1.6 m CD at 05:00 hr of the following morning (Figure 2.10B).

2.3.1.4 July 2010

In late July 2010, spawning check was carried out on 31 July, 5th night after full moon (28 July). Synchronous spawning of *Porites lutea/ lobata* occurred from 21:40 to 22:15 hrs. Male colonies were observed to release clouds of sperm at 21:40 to 22:00 hrs, and female colonies released positively buoyant eggs at 22:05 to 22:15 hrs (Figure 2.13). The water temperature was 30.2℃ at 21:30 hr (Figures 2.9A, D) and coral spawning occurred in an incoming tide near the lower of the semi-diurnal high tide level of +1.4 m CD (Figure 2.10C). The highest tide was at 02:00 hr, reaching +1.6 m CD.

2.3.2 Fertilization and *Platygyra acuta* larval rearing

The spawning time for *Platygyra acuta* was from 21:30 to 22:00 hrs on 5 June, 2010. Gametes from nine coral colonies were collected. Fertilization started at 23:25 hr,

with sperm concentration of around 10^7 ind./ ml. Fertilization rate was 95.04% and up to two hundred thousand larvae were obtained (Table 2.2). Temperature ranged from 22.2 to 31.7°C (average 26.12 ±1.8°C) throughout the culturing period. Coral larvae started to rotate 12 hrs after fertilization and became highly mobile 48 hrs after fertilization. Majority of the larvae showed competent behavior four days later.

2.3.3 Embryonic development of *Platygyra acuta* larvae

Different early developmental stages of *Platygyra acuta* were examined, from egg-sperm bundles, fertilized eggs to planula stage. Eggs and sperms were compressed and released from the polyp of *P. acuta* as egg-sperm bundles (Figure 2.14). The first cleavage was initiated at 2 hr after fertilization. Cleavage split the egg symmetrically, creating a heart-shaped zygote (2-cell stage) (Figure 2.15). Four equally sized blastomeres (4- cell stage) were observed at 3 hr after the second cleavage. The third cleavage was perpendicular to the first two cleavage planes, creating eight blastomeres (8-cell stage) that further divided into 16 blastomeres (16-cell stage) at 4 hr. Synchronous cleavage was gradually lost in both 8-cell and 16-cell stages. After 16-cell stage, the blastomeres continued to divide irregularly. Smoothening of the embryo surface was observed at 8 hr, embryos became flattened and bowl shaped. After 16 hr, the blastula gradually thickened and became spherical

in shape. Larvae began to develop cilia and stated to rotate at 12 hr. They elongated and developed into highly mobile planulae after 48 hrs (Figure 2.15).

2.3.4 Induced settlement

Up to 120,000 larvae were induced to settle on 420 tiles in settlement tank for three days. Temperature of the settlement tank ranged from 26.4 to 28°C (average 27.41 ± 0.47°C) through out the settlement period. Total number of coral spats (settled larvae) recorded was 8446 and the settlement rate was 6.83% (Table 2.3).

2.4 Discussion

2.4.1 Spawning observation

Hong Kong, located at the northern limit of Southeast Asian region with winter seawater temperature that drops to 14-16°C, has a marginal environment for reef building scleractinian corals. However, corals can still successfully reproduce in this less than hospitable environment. In Hong Kong, majority of the coral colonies spawn during the main spawning months from May/June to June/July (Lin, 2003; present study). A total of 12 species from eight genera and three families of scleractinian corals were observed to release gametes in 2009 and 2010 in TPCMP. Eleven species were hermaphroditic, and one species was gonochoric. Multi-specific

synchronous spawning by two or more species was observed occurring during one or a few nights in May 2009, June and July 2010. This spawning pattern is consistent with that observed in many Indo-Pacific regions, e.g. Japan, Taiwan, the Philippines, Singapore and Thailand (Dai *et al.*, 1992; Hayashibara *et al.*, 1993; Guest *et al.*, 2005; Vicentuan *et al.*, 2008; Kongjandtre *et al.*, 2010; see also review by Harrison, 2011).

In these two years of spawning observation, split-spawning was observed in populations of some coral species. Their spawning pattern was synchronous within the same night but spawning continued over consecutive nights and lunar months by the same or different colonies, e.g. spawning of *Platygyra acuta* and *P. carnosus* after full moon in May 2009, May and June 2010. This spawning pattern has also been reported from other regions, e.g. Australia (Willis *et al.* 1985; Babcock *et al.*, 1986; Oliver, 1992), Singapore (Guest *et al.*, 2005), Taiwan (Dai *et al.*, 1992) and Japan (Hayashibara *et al.*, 1993). Split spawning was observed over two consecutive nights in June 2010 in a tagged *P. acuta* colony and has likely occurred in other colonies as well. Split-spawning is thought to be a reproductive strategy that minimizes the risk of catastrophic event (Richmond and Hunter, 1990) or unfavorable environmental conditions like salinity change in sea surface due to heavy rain that could destroy the annual reproductive efforts of a species. It provides

a second chance for colonies that were not fully reproductive or were under stress (Van Veghel, 1994). The causal factors for this strategy, however, are unclear. Further observations are needed to confirm the extent of split-spawning within or between colonies as a response to uncertainty in natural environmental fluctuations or even to human disturbances.

One thing should be noted that the coral spawning observation is limited to the study site and also by limited number of people. Therefore, it is likely that a number of other spawned coral species could have been missed, especially if these are uncommon or are more sparsely distributed. The timing of observation was also limited to early evening after sunset. Species that spawn after midnight or in early morning would have been missed as well. The actual number of species that spawn during these observation periods is likely to be underestimated. However, by definition, these species would not have been involved in mass spawning. At best, they would simply be part of the roster of species that spawn in this multi-specific spawning event. On the other hand, the synchronized spawning among different colonies of the same species, e.g. *P. acuta*, in a mass spawning event has been well documented in the present study. It is the most detailed documentation of such event in southern China thus far.

In Hong Kong, the substantial overlap in spawning time between different species during multi-specific synchronous spawning event suggests the potential for possible hybridization among species (Richmond, 1997; Willis *et al.*, 1997), e.g. *P. acuta* and *P. carnosus*. Further studies of self-compatibility in *Platygyra* species are needed to verify if these phenotypically highly variable species are capable of hybridization. This may also help to ascertain the status and validity of these species.

2.4.2 Embryonic development of *Platygyra acuta*

Embryonic development of Acroporids is well described in the literature (e.g Nami and Motokawa, 2007) as they are often the species used in restoration works (Guest *et al.*, 2011). The present work is the first detailed documentation of embryonic development of *Platygyra acuta*, a Faviid. In general, the developmental stages are similar to those described for Acroporids except that a very thin single layer prawn-chip stage was not observed. It is possible that this stage may be very short in *P. acuta*, and may have been missed. The rate of development was generally comparable to that found among the Acroporids.

2.4.3 Environmental cues

In TPCMP, coral spawning event seems to be closely linked with some environmental conditions, in particular, seawater temperature, tide level and moon phases. Spawning events observed started in May at a time when seawater temperature was rising. In Hong Kong, annual variation in monthly average seawater temperature is 12/13°C, with peaks occurring from June to October and an annual low from December to April. Therefore, the beginning of spawning in May 2009 and June 2010 suggested that seawater temperature may act as a seasonal cue for its onset. This is consistent with previous findings from other regions that spawning period of most of the coral species occurs when water temperatures are rising or when it is the warmest time of the year (Harrison and Wallace, 1990; Richmond and Hunter, 1990). In the present study, the first coral spawning took place on the seventh and fourth night after full moon in May 2009 and 2010, with seawater temperature being 25.9°C and 25.7°C respectively. This suggests that the threshold temperature for coral spawning in Hong Kong might be around 26°C.

In spite of this, there was an apparent difference in the timing of the first main spawning event in the two years of study, being May in 2009 and June in 2010. This revealed that other than rising temperature, there was also a close relationship

between spawning date and lunar phase. Full moon in May happened to be in early May (9th May) in 2009 and in late May (28th May) in 2010. Therefore, by following lunar cycle as a spawning cue, spawning would occur in May in 2009 and in June in 2010. This appears to support the suggestion that seawater temperature acts as a proximate seasonal cue and lunar phase provides the fine tuning for spawning to occur in a particular night or nights (Shlesinger and Loya, 1985; Harrison and Wallace, 1990; Richmond and Hunter, 1990, Richmond, 1997).

In the two years of spawning observation, spawning consistently occurred between 19:20- 22:00 hrs, during/close to the lowest tidal level of the day (except in late June 2010) when tidal mixing would be minimal (difference in tide height <0.5m). It has been suggested that low water volume and water motion during low tide may enhance fertilization success by minimizing the sperm dilution effect for broadcast spawning corals (Babcock *et al.*, 1986; Oliver and Babcock, 1992). These findings suggested that tidal regime after full moon may have played an ultimate role in determining the nights and time of coral spawning. There are, however, some exceptions to this observation. Spawning time in Akajima, Japan occurred around high tide, suggesting that this may be a different strategy that favors the dispersal of gametes over fertilization success in controlling spawning time (Hayashibara *et al.*,

1993). Since spawning consistently occurred between 19:20- 22:00 hrs, onset of darkness has also been suggested as the final cue to determine the hour of spawning (Babcock *et al.*, 1986).

2.4.4 Fertilization, larval rearing and induced settlement

The overall fertilization rate (95.04%) is comparable to that suggested in the Reef Restoration Manual (Guest *et al.*, 2011) that >90% fertilization success can be achieved. The settlement rate (6.83%), however, was very low. It is believed that the larvae might not yet be fully competent during the induced settlement process. Therefore, competency test should be conducted, rather than simply observing the competency behavior of the larvae, in order to have a more precise handle on the appropriate time to start the induced settlement process.

2.5 Summary

Hong Kong, located at a sub-tropical region with colder seawater temperature and high turbidity, is a marginal environment for reef building scleractinian corals. However, corals can still successfully reproduce in this inhospitable environment. This study is the first detailed documentation of spawning behavior of corals in Hong Kong. While only 12 out of a total of 84 known species of corals in Hong Kong were

observed to spawn in the present study, these 12 species represented nevertheless some of the most common and dominant species in Hong Kong. This study also marks the first time the successful collection of egg-sperm bundles from the targeted species *Platygyra acuta*, detailed observation of their fertilization and developmental process, larval rearing and their induced settlement on artificial substrata in Hong Kong or elsewhere in the world.

Natural coral recruitment rate is very low in TPCMP (see Chapter 3 for details). If recruitment failure is consistent, it will take a very long time for any coral community to recover naturally after a destructive event. A case is already in point wherein part of the core area in AYW, TPCMP was destroyed by a barge during a storm in 2006 and until now, no recovery of any degree could be observed (Tam and Ang, 2010). Under such circumstances, techniques to culture corals through larval rearing, either by introducing competent larvae or settled coral recruits back in the field, may provide an alternative to help in any future restoration work for Hong Kong coral communities.

Restoration using sexually produced propagates is preferred over the use of asexually produced coral nubbins (fragments). The former could provide a higher genetic

diversity, a larger number of juvenile corals, and would impose the least damage on donor coral colonies (Guest *et al.*, 2011). However, larval rearing is much more labor intensive and expensive when compared with the asexual methods. It also requires expertise and background knowledge of the reproductive patterns of the target coral species. But Hong Kong coral communities are usually very small. They cannot afford to serve as a donor site for coral nubbins. In the end, larval rearing approach, despites its limitations, may be the only option available for Hong Kong.

The success in obtaining coral larvae and in inducing them to settle in the present study provided some baseline information for future application of larval rearing approach as a way to develop restoration strategy for Hong Kong coral communities. Most other restoration works involving larval rearing focus only on a few species, mainly members of the genus *Acropora* and few members of the family Faviidae (Guest *et al.*, 2011). This present work is therefore significant as it contributes more information about an important member of the Faviidae, *Platygyra acuta*, especially its spawning seasonality, embryonic development, larval rearing and competence. Restoration using a local species is always advisable, despite the fact that massive corals like *P. acuta* are slow growing. It being the most dominant species in Hong Kong cannot simply be a coincidence. No doubt it is well adapted to Hong Kong

environment, making it the logical choice as a target species for restoration works. Larvae obtained from coral culture using sexual reproduction may also be used in many other studies to facilitate a new range of investigations on coral early life stages. It will be critical to examine the adaptation or susceptibility of coral early life history stages to different environmental changes, and to understand how these adaptations could eventually be translated into part of the coral population and community dynamics. Further studies to include other coral species in Hong Kong, e.g. the faster growing *Acropora* spp. and other dominant species, could also be a direction that is worth exploring in the near future.

Table 2.1 Characteristic of corals observed to have spawned in the field in May 2009 and May to July 2010 from TPCMP. Symbol- sex: H, hermaphroditic, G, gonochoric. Mode: S, broadcast spawner. Timing of spawning: nights after full moon when spawning was observed, with full moon date in ( ).

Family and species	Sex	Mode	Timing of spawning: Nights after full moon with full moon date in ( )
Family Acroporidae			
Acropora digitifera	H	S	8 (26 June, 2010)
Aropora tumida	H	S	7 (28 May, 2010); 8 (26 June, 2010)
Family Faviidae			
Cyphastrea chalcidicum	H	S	8 (28 May, 2010)
Favia rotumana	H	S	7-8 (9 May, 2009); 6-8 (28 May, 2010)
Favites abdita	H	S	7 (26 June, 2010)
Favites acuticollis	H	S	7 (26 June, 2010)
Favites pentagona	H	S	7 (26 June, 2010)
Goniastrea aspera	H	S	9-10 (28 May, 2010); 6-7 (26 June, 2010)
Leptastrea purpurea	H	S	8 (26 June, 2010)
Platygyra acuta	H	S	7-8 (9 May, 2009); 7-10 (28 May, 2010), 7-8 (June, 2010)
Platygyra carnosus	H	S	7-8 (9 May, 2009); 7-8 (May, 2010), 8 (June, 2010)
Family Poritidae			
Porites lutea/ lobata	G	S	5 (July, 2010)

Table 2.2 Detailed information on the timing of spawning, fertilization rate calculation and number of larvae that successfully developed from the fertilization and larval rearing experiments.

Species	Spawning		Fertilization time (hr)				No. of	Water vol. at	Fertilization	Total no.
	date	time (hr)	start	1st wash	last wash	count	colonies	fertilization (ml)	rate (%)	of larvae
Platygyra acuta	5-Jun-10	21:30 - 22:00	22:36	23:36	23:57	01:36	9	20,000	95.04	215,333

Table 2.3 Detailed information on the induced larval settlement experiment.

Species	No. of larvae	Vol. of water (L)	Type of substratum	No. of Tiles	Total area of tiles (cm^2)	Total no. of settlement counted	Density of polyps (polyps/cm^2)	Settlement rate (%)
Platygyra. acuta	123,733	1,000	1.5 months conditioned Small ceramic tiles (size = 44 cm^2)	260	44 x 260 = 11,440	3074	0.269	6.83
			1.5 months conditioned Ceramic tiles (size = 117 cm^2)	160	117 x 160 = 18,720	5372	0.287	

Figure 2.1 Pre-set bundle collector (a funnel shaped 450 μm plankton net with a semi-transparent bottle attached at the mouth) on top of a tagged coral colony, *Platygyra acuta*, in Tung Ping Chau Marine Park, Hong Kong.

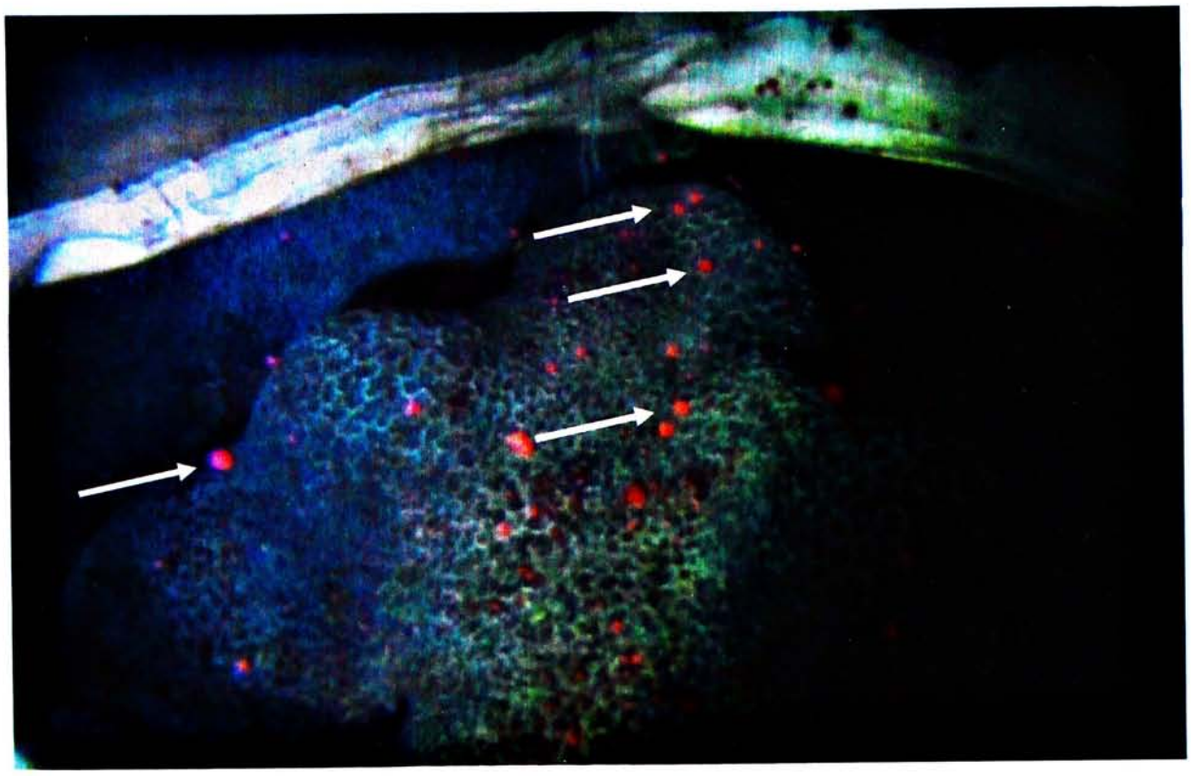


Figure 2.2 Positively buoyant egg-sperm bundles (arrows) released from a *Platygyra acuta* colony being trapped by the bundle collector.

Figure 2.3 A make-shift laboratory set up in Tung Ping Chau Marine Park, Hong Kong.

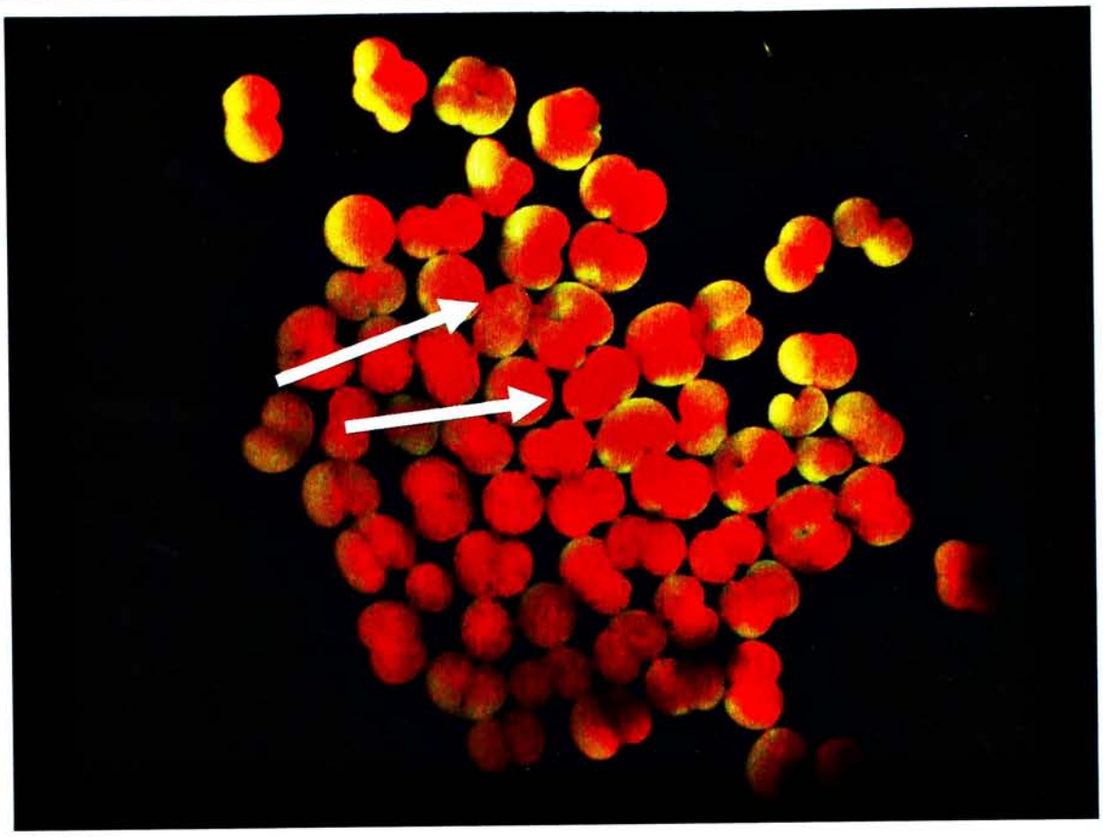


Figure 2.4 Eggs 3 hrs after fertilization. Eggs which appeared in multiple-cell stages were considered as fertilized, while unfertilized eggs remained as round (arrows).

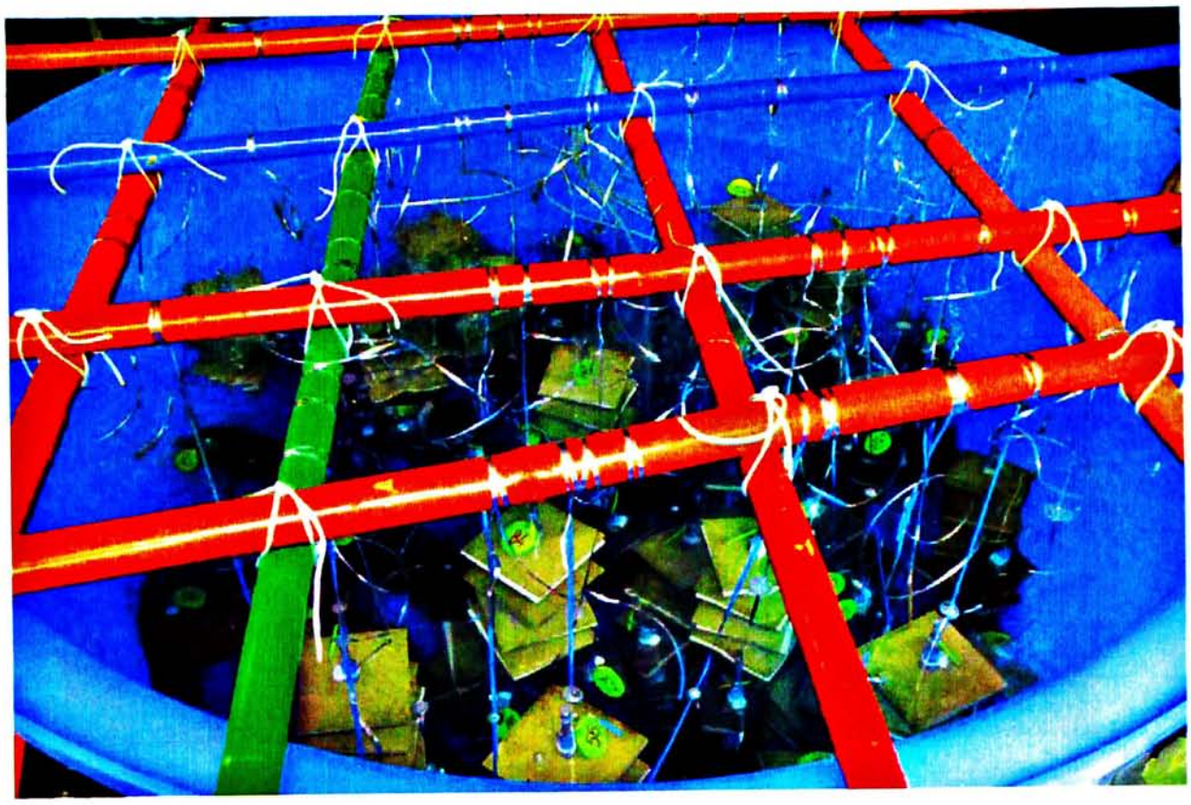


Figure 2.5 Settlement tank with tile rods hanging vertically to expose the ceramic tiles to competent coral larvae.

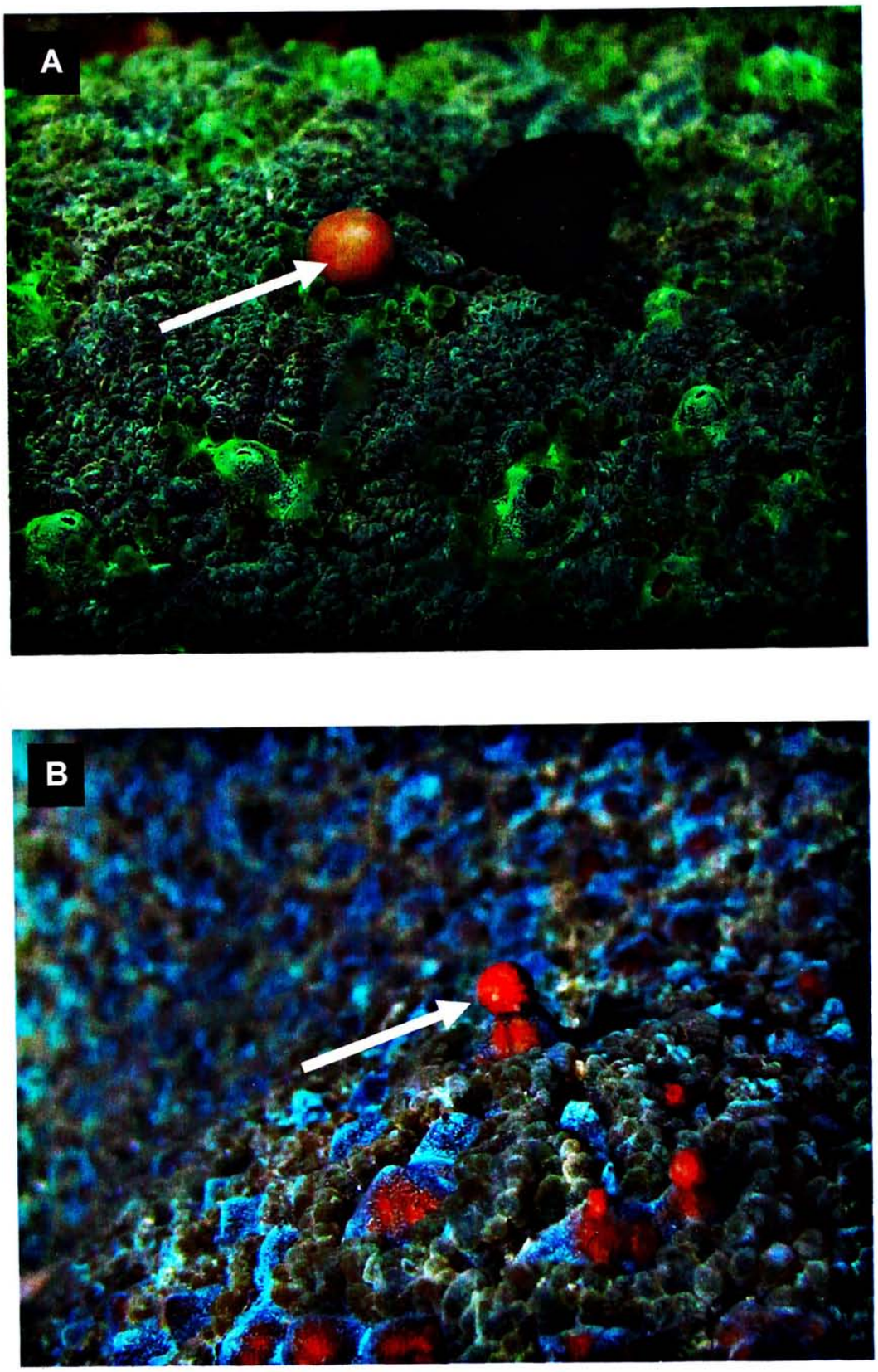


Figure 2.6 Hermaphroditic coral (**A**) *Favites pentagona* (**B**) *Platygyra acuta* releasing discrete, compact egg-sperm bundles (arrow) at night in Tung Ping Chau Marine Park, Hong Kong.

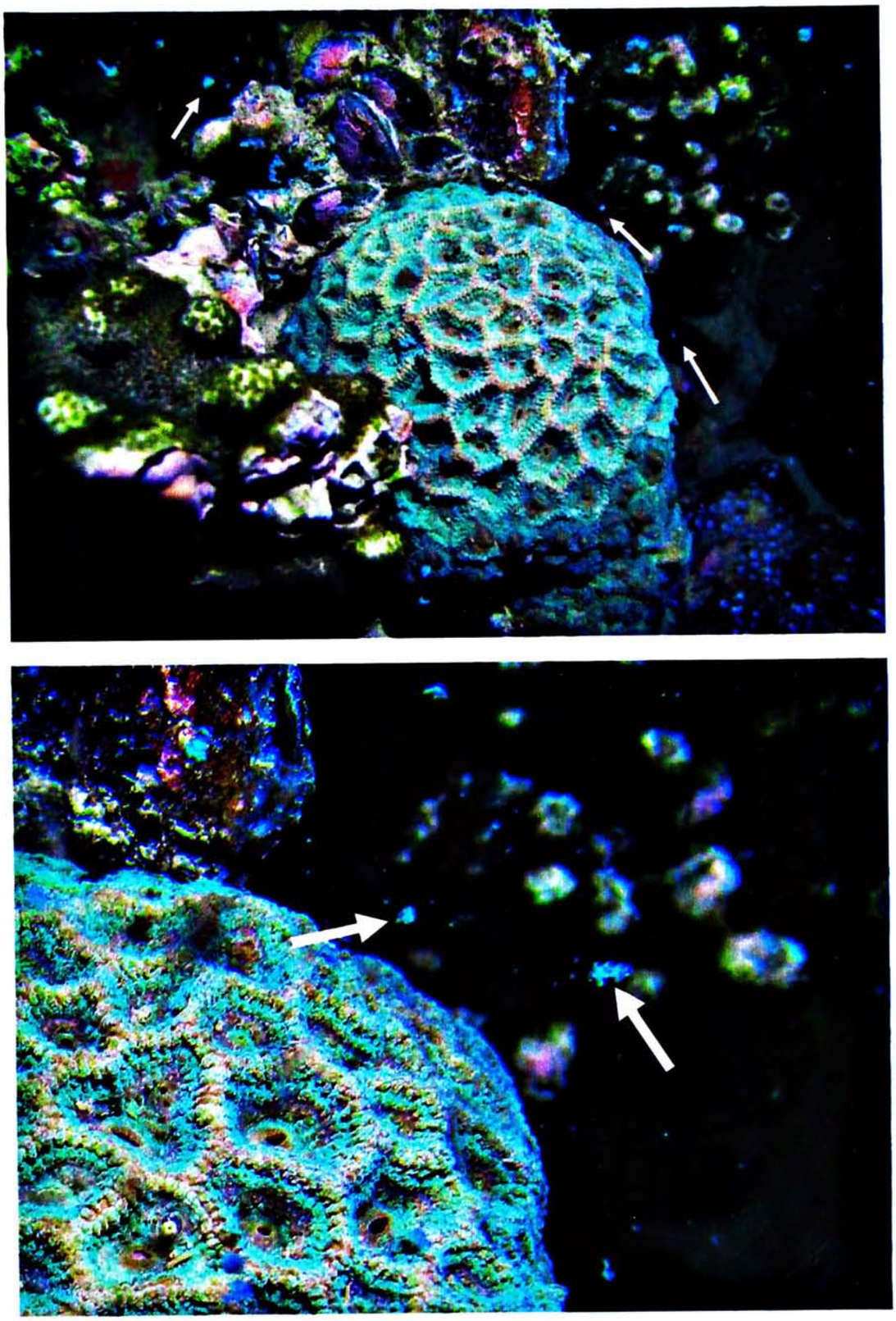
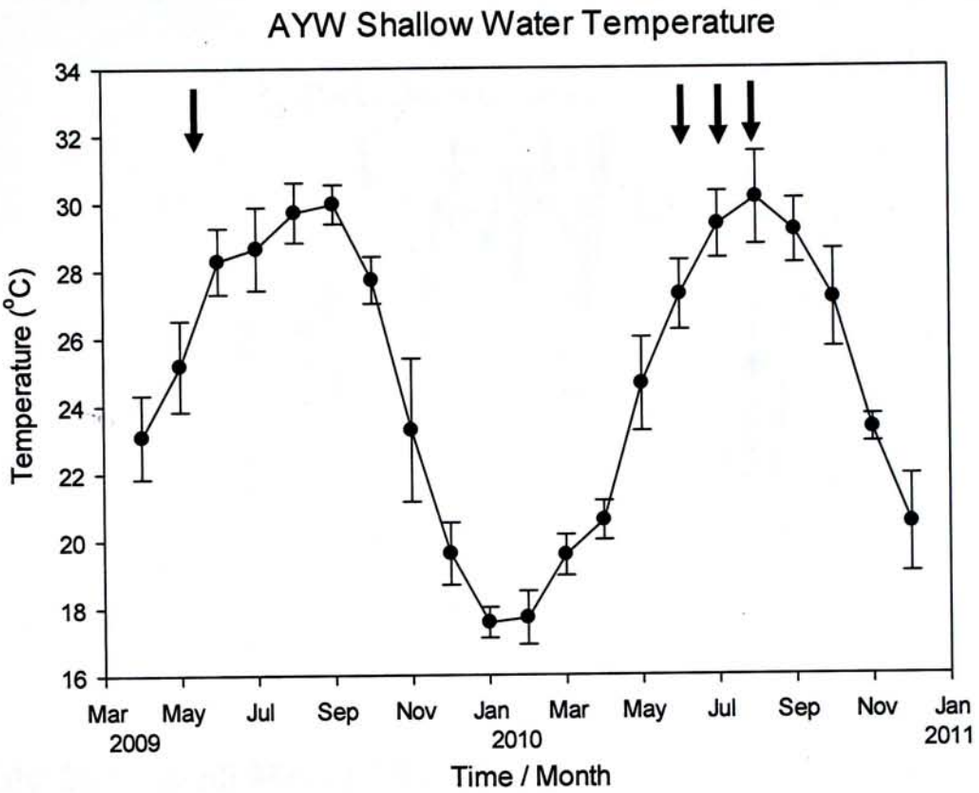


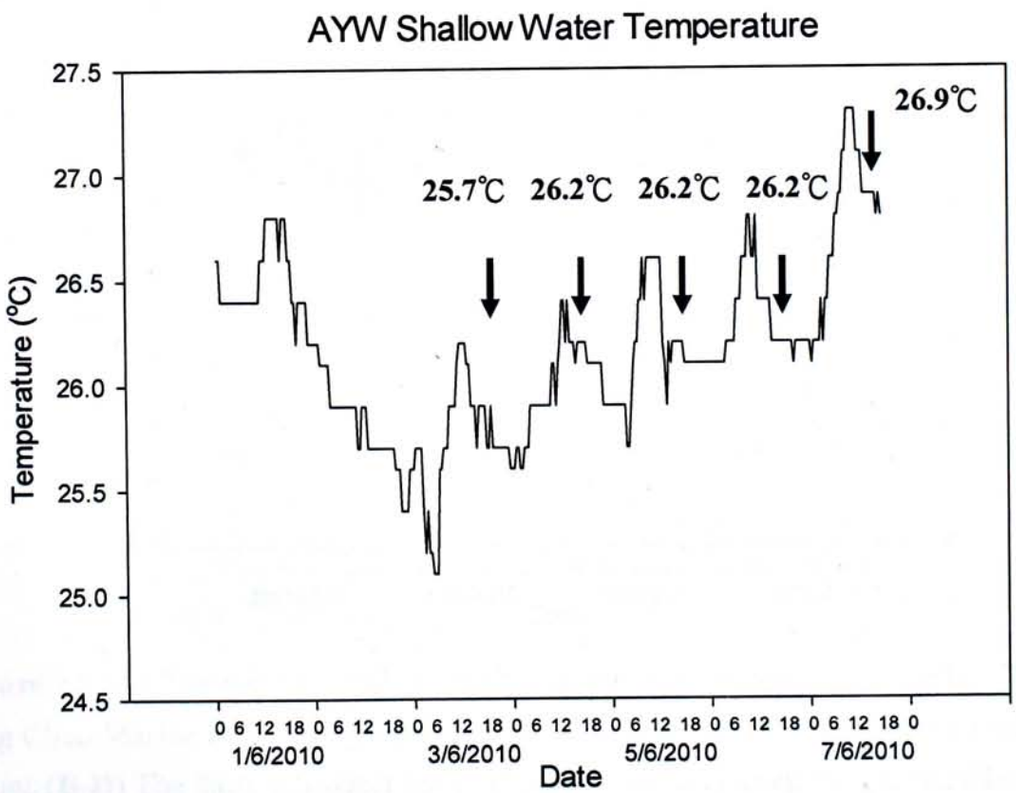
Figure 2.7 Hermaphroditic coral *Goniastrea aspera* releasing loosely bounded grey color bundles (arrows) at night in Tung Ping Chau Marine Park, Hong Kong.

Figure 2.8 Photograph taken from underwater showing surface slick resulting from mass spawning event at 19:30 hr, 16 May 2009 in Tung Ping Chau Marine Park, Hong Kong.

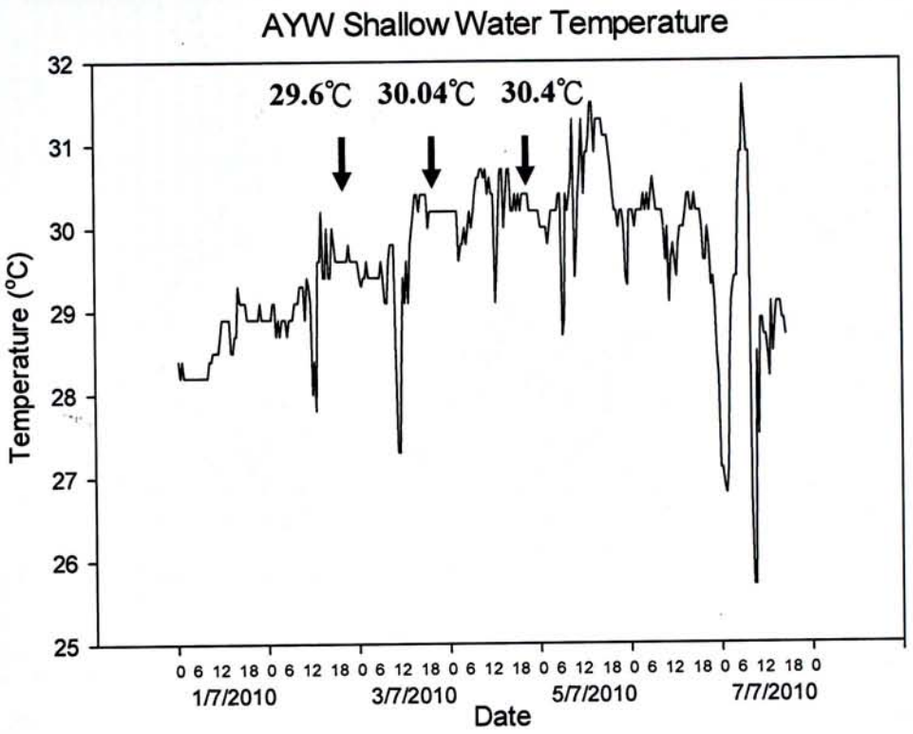
A. Monthly mean seawater temperature (2009-2010)



B. May 2010 (Full Moon 28 May)



C. June 2010 (Full Moon 26 June)



D. July 2010 (Full Moon 26 July)

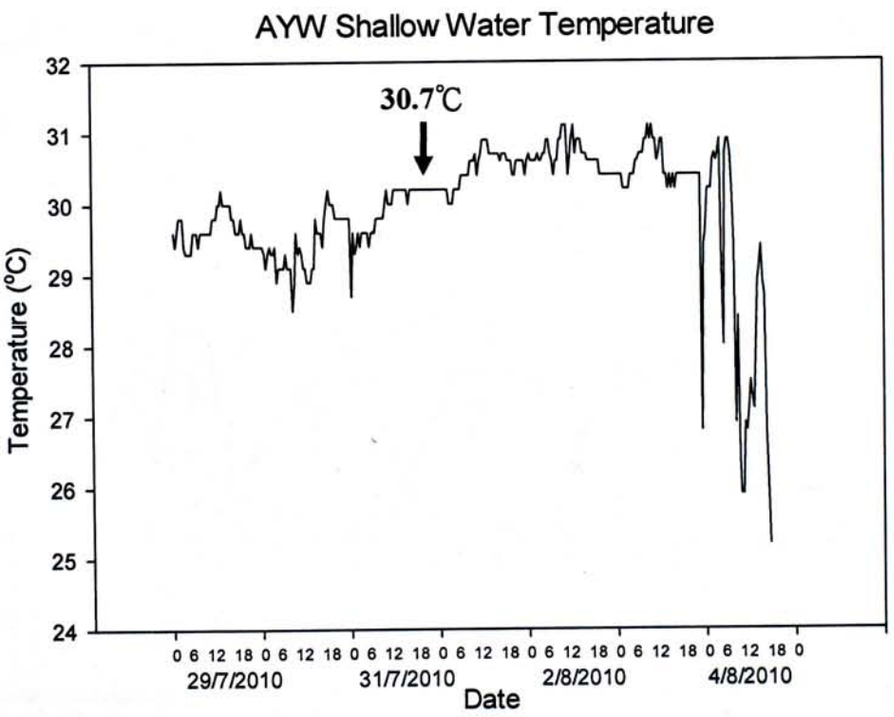
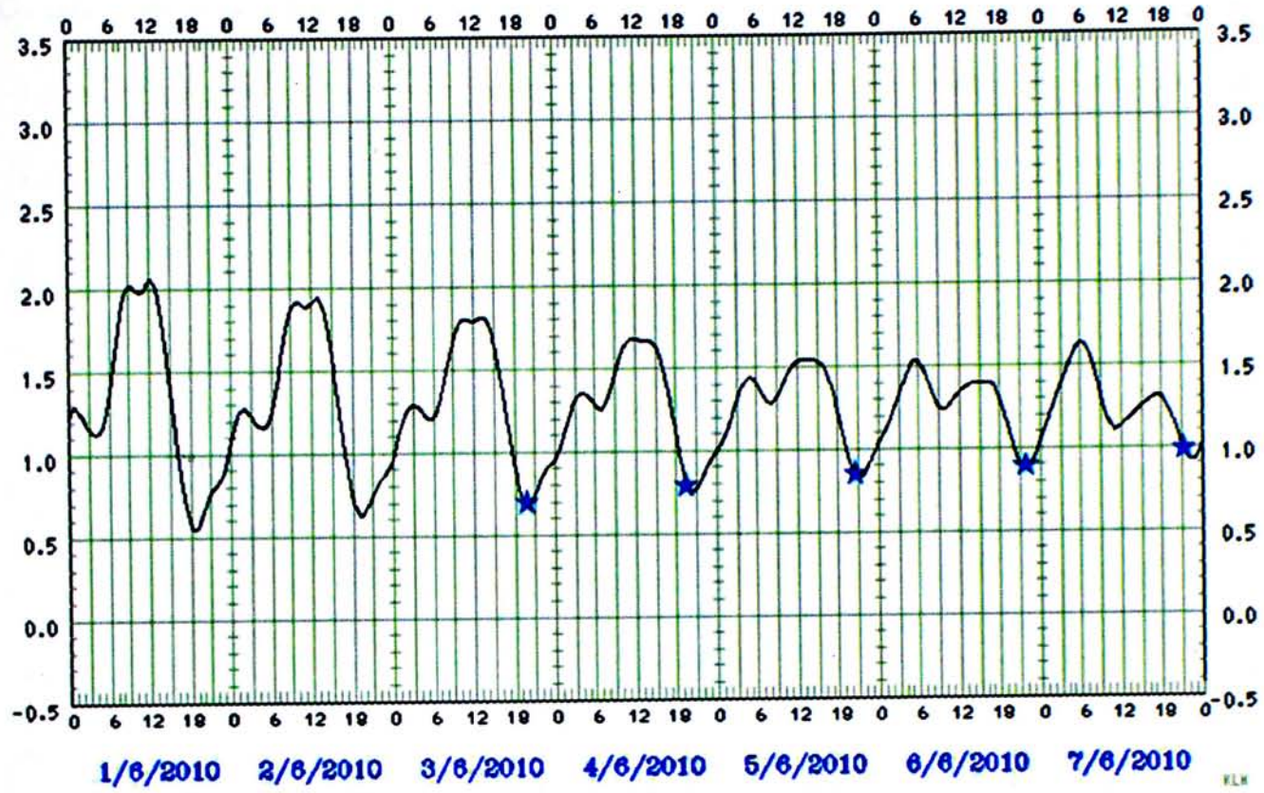
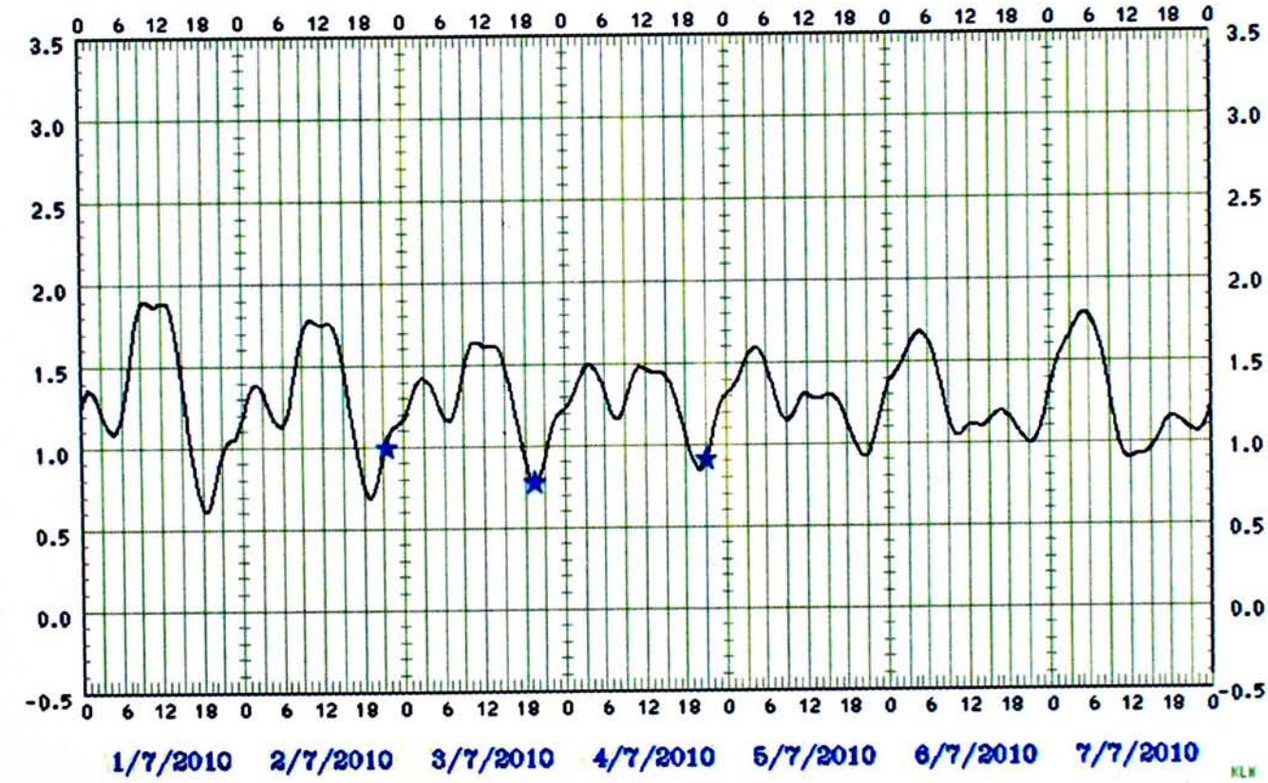


Figure 2.9 (A) The mean (± SD) monthly sea water temperature recorded in Tung Ping Chau Marine Park, Hong Kong. Spawning events observed are marked with an arrow. **(B-D)** The daily sea water temperature recorded in Tung Ping Chau Marine Park, Hong Kong during the spawning events in 2010 (arrows).

A. May 2010 (Full Moon 28 May)



B. June 2010 (Full Moon 26 June)



C. July 2010 (Full Moon 26 July)

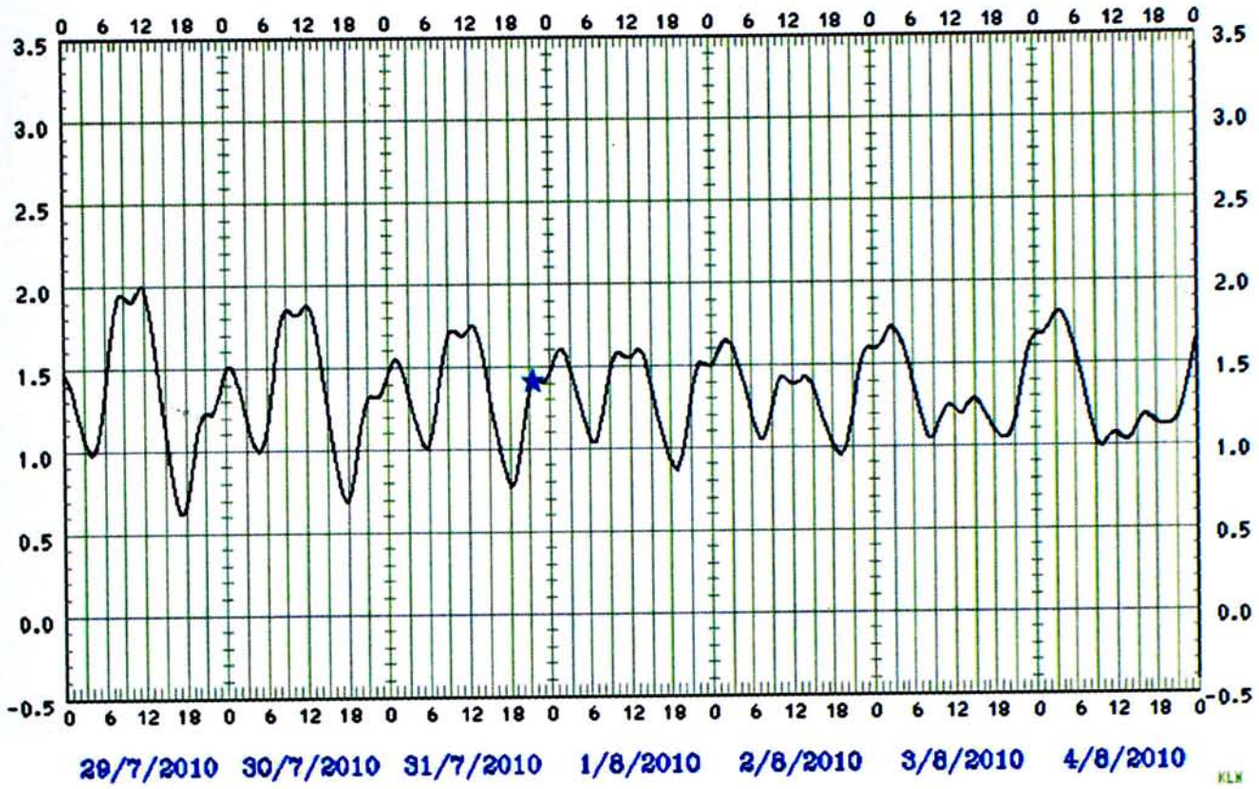


Figure 2.10 Tidal level during period of spawning in May to July, 2010. Spawning events observed are marked by (★)

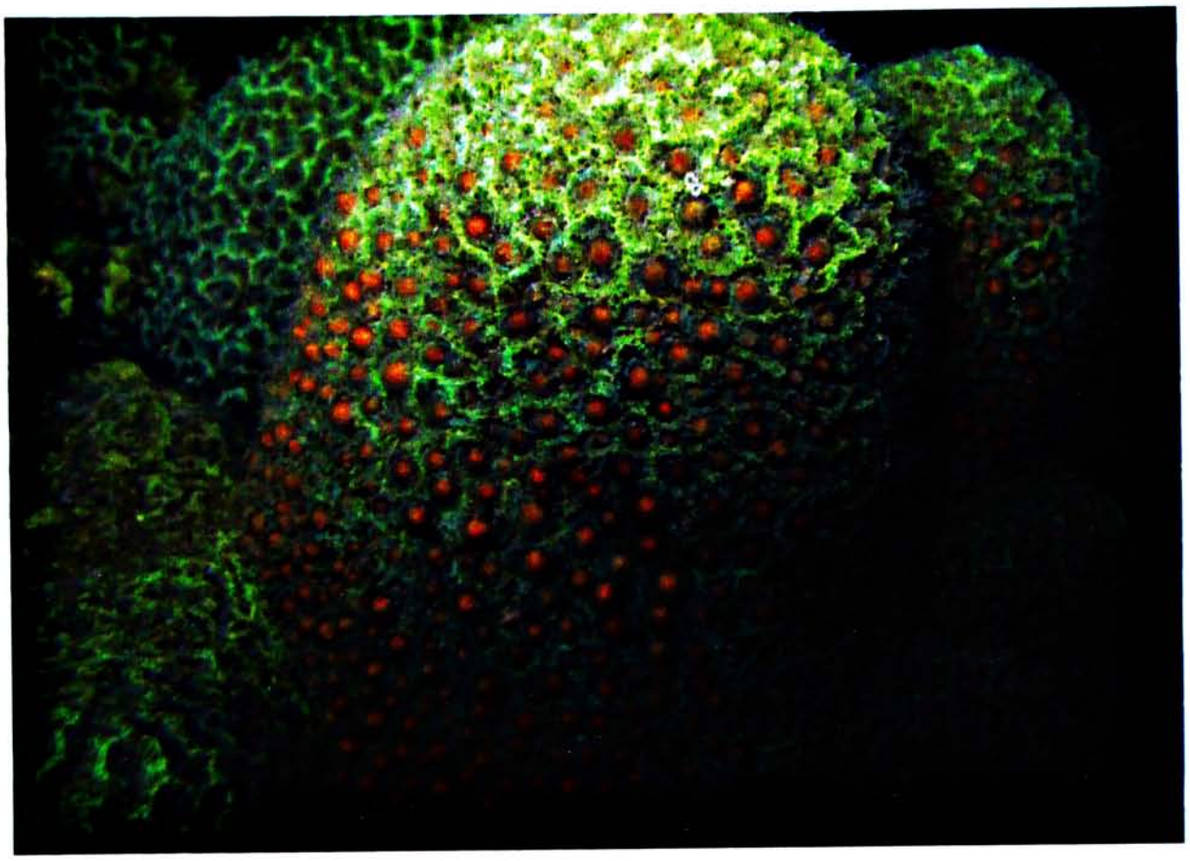


Figure 2.11 "Setting" stage of *Platygyra acuta* with egg-sperm bundles appearing below the oral disc of the polyps at night in Tung Ping Chau Marine Park, Hong Kong.

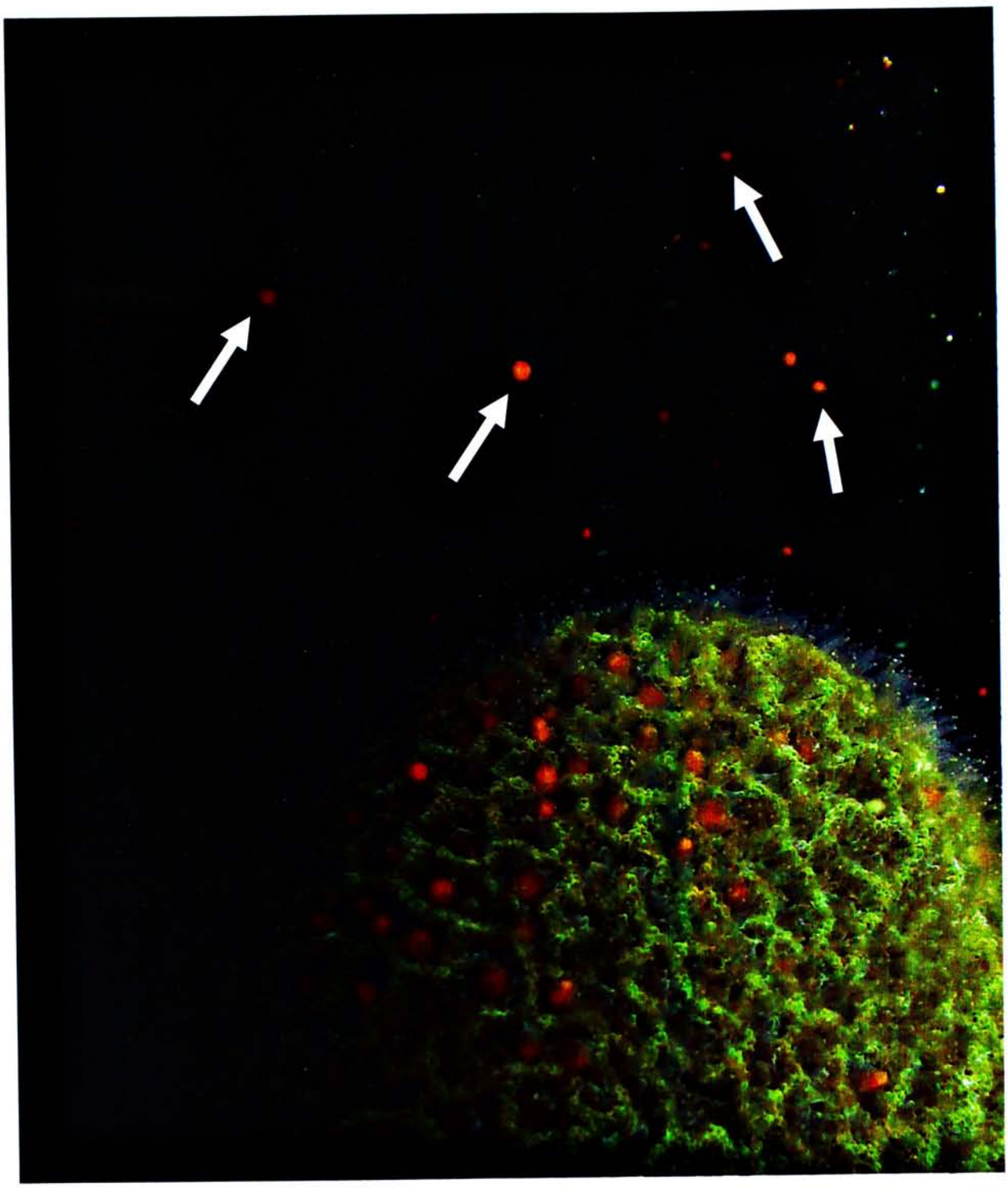


Figure 2.12 Hermaphroditic massive coral *Platygyra acuta* releasing egg-sperm bundles (arrows) at night in Tung Ping Chau Marine Park, Hong Kong.

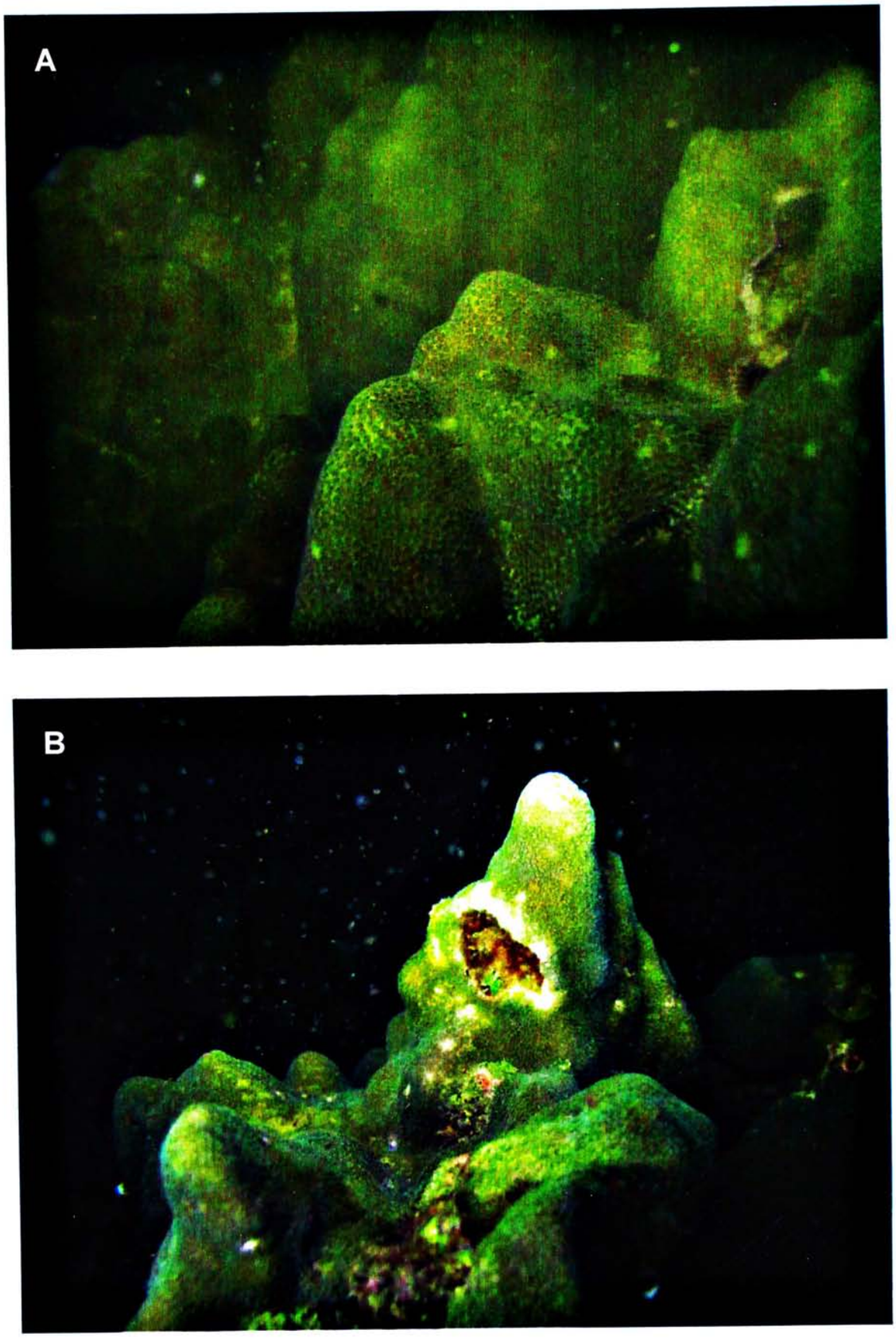


Figure 2.13 (A) Male and **(B)** female colonies of (*Porites lutea/ lobata*) releasing clouds of sperm and eggs respectively at night in Tung Ping Chau Marine Park, Hong Kong.

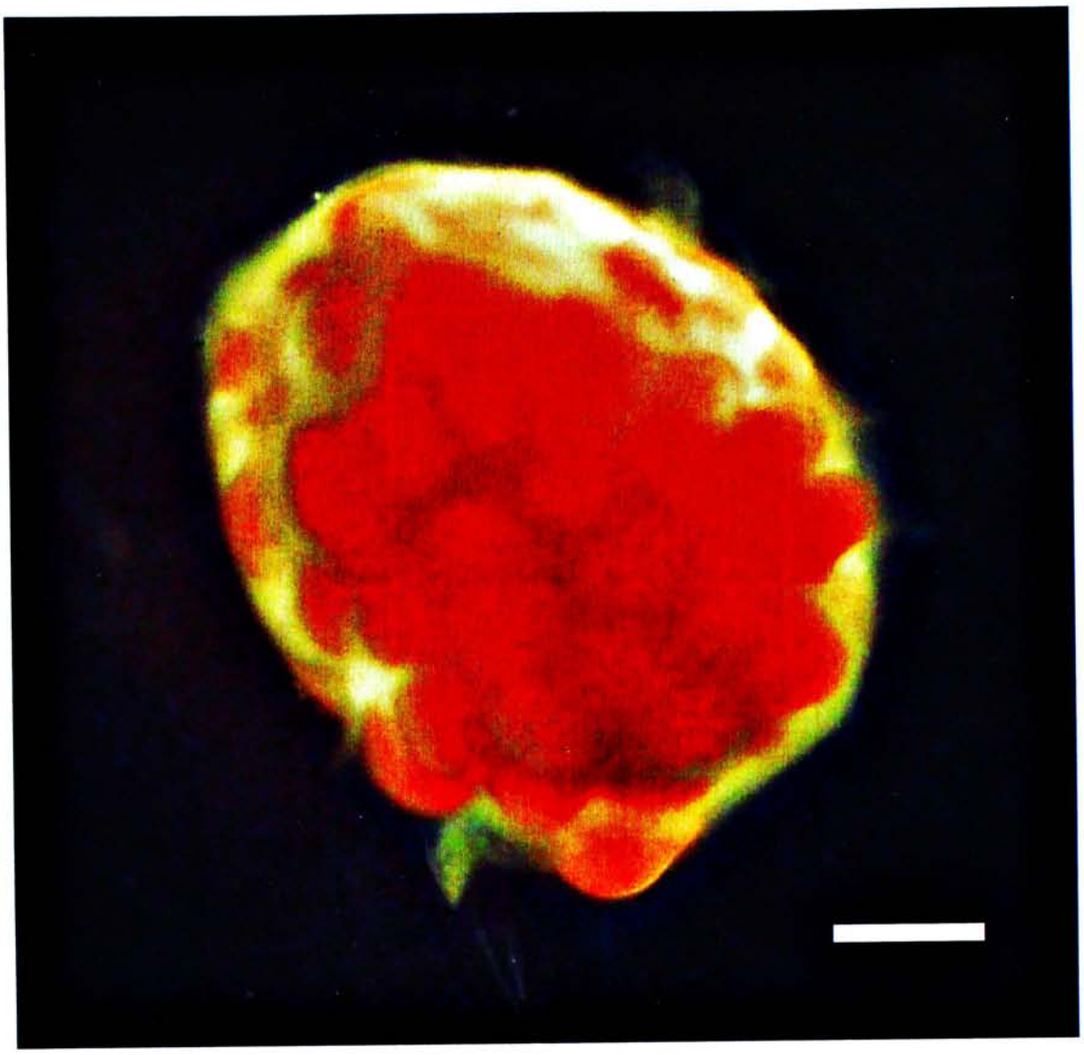


Figure 2.14 Egg-sperm bundle of *Platygyra acuta* immediately after being released. Scale bar=500 μm.

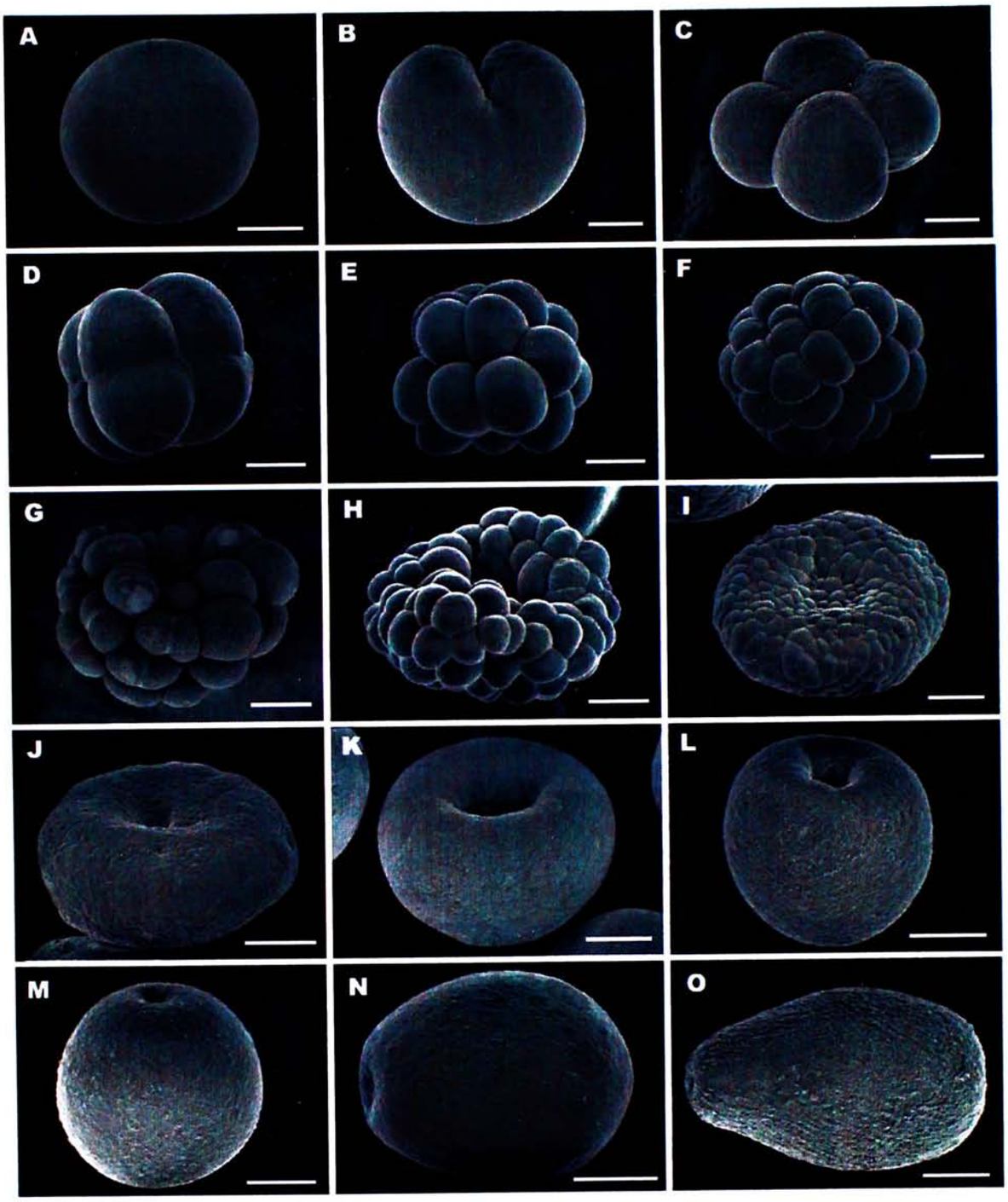


Figure 2.15 SEM photograph of embryonic developmental stages of a broadcast-spawning scleractinian coral, *Platygyra acuta*. (**A**) Egg, where fertilization may or may not have occurred. (**B**) 2-cell stage (2 hr). (**C**) 4-cell stage (3 hr). (**D**) 8-cell stage (4 hr). (**E**) 16-cell stage (4 hr). (**F**) 32-cell stage (5 hr). (**G-H**) Bowl stage (5 hr, 6 hr). (**I-J**) Smoothening of cell surface (8 hr, 12 hr). (**K-M**) Blastula stage (16 hr, 24 hr, 32 hr). (**N-O**) Elongated stage (36 hr, 96 hr). Scale bar=100 μm.

Chapter 3

Recruitment Patterns of Scleractinian corals in Tung Ping Chau Marine Park, Hong Kong

3.1 Introduction

The importance of coral recruitment as a key process in the maintenance of coral communities and in facilitating reef recovery after disturbance is well recognized (Johnson and Preece, 1992; Connell, 1997). Recruitment refers to the point at which new juvenile coral 'recruit' becomes visible to census (Keough and Downess, 1982; Connell, 1985; Harrison and Wallace, 1990). Before that, a competent coral larva must attach itself to the substratum and metamorphose into a juvenile polyp 'spat'. The number of settlement and post-settlement survival of coral recruits need to be monitored over time to provide the baseline information about coral recruitment patterns. This information is essential as a basis for early warning of potential damage to coral community or assessment on its recovery after disturbance (Bellwood *et al.*, 2004). This, in turn, could help towards the design of effective management strategies for the conservation of coral reefs.

Ex situ microscopic examination of removable settlement tiles has been used widely

to study coral settlement and recruitment patterns (Harrison and Wallace 1990). Conventional census techniques under day light are used to detect and monitor the coral recruits *in situ*. However, newly settled corals are very small, often ≦2 mm in diameter, and their growth is slow (Babcock, 1985; Babcock *et al.*, 2003). Because of their cryptic settlement behavior and small size, they are difficult or impossible to detect by naked eye. It may take up to a year for a coral recruit to become easily observable *in situ* (Wallace and Bull, 1981). During this early stage of post-settlement, mortality is typically high (Babcock, 1985; Wilson and Harrison, 2005). Therefore, this time lag between settlement and detection of recruits makes it difficult to estimate survival of early stages of new recruits in the field (Baird *et al.*, 2006).

The development of florescence census technique recently addresses this problem and allows early coral recruits to be more easily observed (Piniak *et al.*, 2005; Roth and Knowlton, 2009). The principle of the florescence census technique is to make use of the high abundance of fluorescent pigments in many coral juveniles (Papina *et al.*, 2002). These fluorescent pigments emit blue-green to orange light when exposed to ultra violet (UV), blue or green light (Mazel, 1995). Therefore, by exciting the fluorescent pigments in the recruits' tissues, even very small coral recruits can

become clearly visible (Mazel, 2005, Baird *et al.*, 2006). Under this census technique, up to 97.6% of all coral recruits bigger than 1mm in diameter can be detected *in situ* (Schmidt *et al.*, 2008). This technique can greatly help in rapid estimation of the location and abundance of new recruits *in situ* for more accurate determination of the settlement and early post-settlement survival of corals, and in assisting the estimation of early growth of coral recruits (Baird *et al.*, 2006).

Hong Kong has a sub-tropical climate and low winter temperature (14-16°C) makes it a marginal place for the growth of reef building scleractinian corals. Nonetheless, it is located in southern China, one of the few places in the world where corals could possibly extend their range of distribution should the global warming trend continuous (Hoegh-Guldberg, 1999) Previous researches have focused on the study of current state of the coral communities (reviewed by Ang *et al.*, 2006; Tam and Ang, 2008) showing that under the influence of the freshwater discharged from Pearl River to the west, large region of Hong Kong water, especially the western part, is relatively turbid and low in salinity, hence, is not suitable for coral growth. Therefore, coral communities are mainly concentrated in the eastern and northeastern area of Hong Kong where a true oceanic condition exists and the influence of the Pearl River runoff is least. It is here where high coral coverage of over 60% can be found, for

example, in Tung Ping Chau Marine Park (TPCMP).

However, having high coral abundance and cover in a community does not necessary imply the existence of an efficient recruitment pattern that maintains the community (Hughes *et al.*, 1999). It is therefore important to examine the coral recruitment pattern in such a community in order to understand processes involved in its maintenance. Information on coral recruitment pattern in Hong Kong is largely wanting. To address this knowledge gap, I have initiated a 18-month study that focuses on coral recruitment patterns around two core coral areas, A Ye Wan (AYW) and A Ma Wan (AMW), in TPCMP, where coral diversity is one of the richest in Hong Kong. In particular, three aspects of coral recruitment pattern were addressed:

1. Settlement and post-settlement survival of natural coral recruits on artificial settlement tiles and concrete blocks using fluorescence census technique and microscopic examination.
2. Post-settlement survival of artificially seeded coral recruits of *Platygyra acuta*, the most dominant coral in Hong Kong.
3. Predation effect of gastropods on post-settlement survival of coral recruits.

3.2 Methods and Materials

3.2.1 Site description

Tung Ping Chau Marine Park (TPCMP) (22°32 N, 114°25 E) is an island located in the north-eastern side of New Territories, Hong Kong Special Administrative Region, China. The two coral core areas are located along the eastern side. These sites support high coral coverage of over 60%, with 45 of the 84 scleractinian coral species recorded from Hong Kong (Ang *et al.*, 2003). Corals are most abundant in depths that ranged from -1 m CD (Chart Datum) to -4 m CD. Among the two sites, AMW has a richer coral diversity (Ang *et al.*, 2000). Zonation pattern of coral communities was observed in both sites, with shallow water zone, 0 to -1 m CD, having significantly different coral communities than deeper water zone (> -1 m CD). Substratum for both sites, from 0 to -1 m CD, is mainly composed of rocks; whereas from -1 m to -3 m CD, it is mainly sandy to silty (Tam and Ang, 2007). More details about TPCMP are given in Chapter One (Section 1.4).

3.2.2 Settlement and survival of coral recruits

3.2.2.1 Settlement tiles

To quantify the settlement and post-settlement survival rate of natural coral recruits, monitoring studies on coral recruitment patterns in AYW and AMW were carried out

from May 2009 to October 2010. A total of 24 setups (Figure 3.1) were established at both sites, with 6 setups in each site at each of the two depth ranges (-0.5 to -1.5 m CD and -2.5 to -3.5 m CD). Terracotta tiles were used as artificial settlement plate. Tiles were conditioned in the sites for six weeks prior to the commencement of the experiment. In each set up, three tile pairs were mounted at 45 degrees, the optimal angle for coral settlement (Carleton and Sammarco, 1987), on stainless steel mesh bolted on concrete blocks. Six of these blocks were placed at each of the two depths in each site at 5 m interval from one another. Each block was randomly positioned in different orientation.

In each block, there were three pairs of tiles each designated for a particular purpose. One pair was used for short-term (seasonal) monitoring of coral recruitment and the tiles were replaced every three lunar months. The second pair was used for long-term (one year) monitoring and was replaced after one year. The third pair was for conditioning purpose for use as replacement tiles. All replacement tiles were pre-conditioned for at least six weeks before being used in the experiments. Tiles removed were brought back to the laboratory to be examined under the dissecting microscope for the presence of coral recruits, as well as for an estimate of the cover of other fouling organisms. Each coral recruit found was photographed with a Nikon

P5100 camera mounted on a dissecting microscope for later identification and measurements. Tiles found to have coral recruits were photographed and returned to the field for further observations of recruit survivorship and development. Tiles without any recruit were placed in household bleach for 24-48 hrs to remove organic matters, rinsed, air dried and stored for further examination of the percentage cover of other fouling organisms.

Other than examination of the settlement tiles in the laboratory, fluorescence census technique was used for weekly/bi-weekly monitoring of recruits on all the tiles *in situ* until they were removed from sites. Censuses were conducted during the day. Each time, sediments on the tile surface were first fanned away by hand. The blinking mode of the flash light (FL-1 FLASH Light™, NightSea LLC, California, USA) with an internal blue exciter filter, together with a yellow barrier filter placed over the diver's mask, were then used to facilitate the detection of new recruit on the tile. Once a new recruit was spotted, it was marked on the sketch map of the tile on an underwater writing slate for repetitive observations overtime. All coral recruits on tiles were identified to the family level. Size (diameter and surface area), number of polyps present and condition (i.e. undamaged, damaged, overgrown by other organisms) of each recruit were also recorded *in situ* every week/ bi-week.

3.2.2.2 Concrete blocks

Concrete blocks 46 cm x 30 cm x 15 cm in size formed the base of the experimental setups for mounting settlement tiles. They provided additional surfaces for coral recruitment as well. The horizontal surface of these blocks was therefore also examined under water for the presence of coral recruits on October 2010, after 20 months in the water. Both conventional inspection and the fluorescence census technique were used to locate coral recruits. All coral recruits on these block surfaces were counted and identified to the family level. Size (diameter and surface area), number of polyps present and condition (i.e. undamaged, damaged, overgrown by other organisms) of each recruit were also recorded *in situ*.

3.2.2.3 Fouling organisms on settlement tiles

To investigate the presence of potential competitors of corals for settlement space, percentage cover of major sessile organisms like oysters, tube worms, barnacles and bryozoans on short-term and long-term monitoring tiles were photo recorded after the tiles were placed in household bleach for 24-48 hrs. No attempt was made to identify these fouling organisms beyond the general grouping levels. Images of tiles were taken using a Canon® G10 digital camera and the mean % cover of these organisms on each set of tiles at each site was then calculated using a computer

image analysis program Image-Pro® Plus 6.0.

3.2.3 Environmental parameters

Minilog-T temperature loggers (Vemco, Halifax, Canada) were deployed on one of the setups at each depth from April 2009 to December 2010 in AYW, and from January 2010 to December 2010 in AMW to record the *in situ* temperature conditions at every 30 min interval. Sedimentation traps made of PVC cylinders (15 cm in height and 5 cm in diameter) with opening mounted with mesh (design with reference to Wong, 2001) were also deployed at each depth in each site from November 2009 to January 2011. These traps were replaced every lunar month and brought back to the laboratory for processing. Contents of each trap were poured into a pre-weighed beaker and dried in an oven at 50℃ for one week. The sedimentation rate was calculated based on the following equation and expressed as $mg.cm^{-2}.d^{-1}$.

Weight of sediment = [dry weight (sediment in beaker) – beaker weight]/basal area of sedimentation trap/day of deployment

3.2.4 Post-settlement survival of artificially seeded coral recruits of *Platygyra acuta*

Study on the survival of early stage of *Platygyra acuta* recruits in AYW and AMW was carried out from mid July 2010 to October 2010. Larvae of *Platygyra acuta* were

first induced to settle on ceramic tiles in the laboratory (refer to Chapter Two for detailed description of the methodology). Tiles with known number of young recruits were then taken out to the field and mounted in pairs on the same mesh used to mount the settlement tiles (Section 3.2.2.1). Ceramic tiles were collected after half a month, then every 1.5 months in the next four consecutive months and brought back to the laboratory to be kept in out-door flow-through water table under shade. They were examined under the dissecting microscope for the number of coral recruits still present and returned to the field after a maximum of three days in the laboratory.

3.2.5 Effect of gastropod exclusion on post-settlement survival of coral recruits

Gastropods were often found on the settlement tiles for coral recruits. Predation by them is likely to be one of the main causes of recruit mortality. A study was conducted from mid July 2010 to October 2010 to examine this effect. A total of three set-ups made up of tile rods (Figure 3.2) were established in each of the two depth ranges (-0.5 to -1.5 m CD and -2.5 to -3.5 m CD) in AMW, each 10 m apart in random orientation (Total = 6 setups). Each tile rod had five pairs of ceramic tiles mounted at 5 cm from each other (refer to Chapter Two for more detailed description of this setup). Larvae of *Platygyra acuta* were induced to settle on these tile rods placed together in a big tank for three days. The number of recruits on each ceramic

tile was counted. Tiles with known number of recruits were properly marked, reassembled in different numbered rods and subsequently transferred to the setup in the field. Each setup supported four rods in the following random arrangements: one rod (with 10 ceramic tiles in 5 pairs) is placed in a cage that was fully covered on all sides (Full Cage), one in a cage with the top part open (Top- half Cage), one in a cage with side open (Side-half Cage) and one without any cage (Control). The two half-covered cages served as the cage controls. Tiles were vertically oriented with respect to the rod when placed in the setup to minimize the effect of sedimentation on coral spats. Cages were made of stainless steel mesh of 1 cm mesh size to screen out most of the corallivorous gastropods, *Cronia margariticola* and *Drupella rugosa* (sizes usually ranged from 1.5 to 2 cm and 2.5 to 3 cm respectively).

Tile rods were collected after half a month, followed by every 1.5 months over the total of four consecutive months. Each time, they were brought back to the laboratory submerged in seawater and kept in out-door shaded flow-through water tables. Each ceramic tile was then examined under the dissecting microscope for a count of coral recruits. Tile rods were returned to the field within one week.

3.2.6 Data analysis

Differences in the mean percentage coverage of total as well as the dominant fouling organisms on the settlement tiles at different times in different sites were examined using Two-way ANOVA. Each tile was treated as an independent replicate, with six replicates in each site at each time. Short term tiles were replaced every three lunar months over the period from May 2009 to October 2010, and long term tiles were in the field for one year from May 2009 to May 2010. As tiles were set in pair, one facing up and one facing down, so differences in the mean percentage coverage of the total and dominant fouling organisms between face up and face down tiles at different times were also evaluated using Two-way ANOVA.

Similarly, differences in the post-settlement survival of artificially seeded coral recruits of *Platygyra acuta* in different sites over time were compared using Two-way ANOVA. Each ceramic tile was treated as an independent replicate, with five replicates from AYW and six replicates from AMW. These ceramic tiles were also set in pairs with one facing up and one facing down. Differences in the survivorship of seeded recruits between face up and face down tiles in different sites at each sampling date were also compared using Two-way ANOVA.

Differences in the mean percentages of surviving recruits on tiles in the gastropod exclusion experiment over time were examined using Two-way ANOVA. Each tile rod was treated as an independent replicate, with three replicates for each of the four treatments in each site. This allowed testing of (a) differences in the survivorship of coral recruits among treatments over time, (b) differences in the survivorship of coral recruits among treatments between sites at each sampling date. One-way ANOVA was also employed to evaluate differences in the percentage of surviving recruits on tiles among different treatments in each site at each sampling date.

If significant differences were detected in any ANOVA, then a post-hoc test using Tamhane's test (for equal variances not assumed) was carried out for within- and between-group comparisons. All data were analyzed using SPSS 16.0 for Windows (SPSS Inc., USA).

3.3 Results

3.3.1 Coral recruitment on settlement tiles

3.3.1.1 Total settlement

Short term monitoring (seasonal)

A total of only six recruits (four fluorescent, two non-fluorescent) were recorded on 144 tile pairs from May 2009 to October 2010 at the two study sites. Of these recruits,

one belonged to Family Acroporidae, two were Poritids and one was a Faviid. The identity of the remaining two recruits cannot be ascertained with confidence. A great majority of the tiles did not have any recruit, except for one tile in AYW shallow water that had one recruit, one tile in AYW deep water that had two recruits (Figure 3.3), another two with one recruit on each, and one tile in AMW deep that had one recruit. The number of recruits was very low such that an average of only 0.042 recruits per tile-pair, equivalent to 0.94 recruit m^{-2}, was recorded over the 1.5 years of study.

Long term monitoring (1 year)

The experiment began in May 2009. Only one recruit (fluorescent) was recorded on one of the 48 one-year old tile pairs from May 2009 to May 2010 at the two study sites. The recruit was from Family Poritidae and settled in AYW shallow water, face up tile. It should be noted that the recruit settled on August 2009, within three months after commencement of the study. No new recruits were observed afterwards.

3.3.1.2 Spatial and temporal patterns of coral recruitment

Of the total number of seven coral recruits observed on the short-term and long-term settlement tiles throughout the first year of this study, 4/7 (57.14%) settled on the

face up tiles, while 3/7 (42.86%) settled on the face down tiles. All recruits on face up tiles showed high fluorescent ability (Figure 3.4), while among the three recruits on face down tiles, only one showed weak fluorescent and the rest were non-fluorescent. When comparing between depths, 5/7 (71.43%) of the recruits settled in deeper water, while 2/7 (28.57%) settled in shallow water. Overall, highest recruitment (4/7, 57.14%) occurred at AYW deep water, followed by 2/7 (28.57%) at AYW shallow water and 1/7 (14.29%) at AMW deep water. No recruitment was observed at AMW shallow water. Temporally, majority of the recruits were found on tiles from August 2009 - November 2009 (Figure 3.5). The number of coral recruits was lower during May 2009 - August 2009 and no recruits were observed for the rest of the year. During the subsequent year of monitoring, no recruits were observed on any of the tiles deployed in the sites. Recruitment varied between years, while recruitment in AMW shallow water was consistently zero.

3.3.1.3 Growth and health of coral recruits

All recruits on tiles had only 1 polyp when first detected. The minimum diameter and surface area recorded were 1.03 mm and 0.89 mm^2 respectively for the Poritid recruit. One of the recruits bleached after being brought back to laboratory and eventually died. For the remaining six recruits *in situ*, three (2 unknown, 1 Poritidae) (50%)

were overgrown by barnacle outbreak during November 09 to January 10 (Figures 3.6, 3.7), one (Poritidae) (16.67%) was overgrown by turf algae, one (Acroporidae) (16.67%) just disappeared and only one (Faviidae) (16.67%), located in AMW deep water face down tile, survived until the end of the study period and remained undamaged. Recruits on face up tiles were all observed to be covered by sediment in various degrees during each visit. The maximum diameter and surface area of recruit recorded in these 1.5 years of study were respectively 7.54 mm and 35.36 mm^2 for the Poritid recruit. Poritid grew fastest, had the highest rate of addition of new polyps with the maximum number of polyps recorded being 27 for a four month old colony. Faviid had the lowest rate of addition of new polyps.

3.3.1.4 Competition with other fouling organisms

Oysters, tube worms, barnacles and bryozoans were the major occupiers of space on tiles, their abundance varied among monthly and yearly tiles (Figure 3.8). The mean total % coverage of fouling organisms was significantly different in different sites among time, as well as between face up and face down tiles among times (Two-way ANOVA, $p<0.01$, Figure 3.8). Throughout the study period, the mean total % coverage of fouling organisms in AMW shallow was significantly lower than that in the other three sites (Two-way ANOVA, $p<0.01$; Tamhane's test, $p<0.05$).

Temporally, November 2009 to January 2010 tiles were found to be significantly higher in coverage of biofouling organisms, followed by one year tiles, than the rest of the tiles collected (Two-way ANOVA, $p<0.01$; Tamhane's test, $p<0.05$; Figure 3.8C, G). Among all the tiles monitored, face down tiles in AYW deep water consistently had a greater % coverage of biofouling organisms than the face up tiles, while no such significant differences were observed in other sites (Two-way ANOVA, $p<0.01$; Tamhane's test, $p<0.05$; Figure 3.8).

The high % coverage of fouling organisms from November 2009 to January 2010 was mainly due to a barnacle outbreak that resulted in the mean (± SD) coverage of 89.8 ± 11.66% of the tiles (Figures 3.8C, 3.9). Over the study period, the coverage of barnacles was significantly different between face up and face down tiles over time (Two-way ANOVA, $p<0.05$), but not in different sites (Two-way ANOVA, $p>0.05$, Figure 3.8). Tiles collected from November 2009 to January 2010 had significantly higher coverage of barnacles than the rest of the tiles collected, followed by the one year tiles with mean (± SD) coverage of 79.51 ± 15.15% (Two-way ANOVA, $p<0.05$;Tamhane's test, $p<0.05$; Figure 3.8C, G). However, the difference between face up and face down tiles within any specific site was not as clear (Tamhane;s test, $p>0.05$). No outbreaks of barnacles were observed in the following year (2010-2011).

For the rest of the year, the coverage of biofouling organisms remained constant and ranged from 30-40% on average (Figure 3.8). Oysters (and few other bivalves) abundance varied significantly in different sites, as well as between face up and face down tiles over time (Two-way ANOVA, $p<0.01$). Tiles from July to October 2010 (Figure 3.8F) had significantly higher % coverage of oysters than the rest of the tiles collected, except those from May to August 2009 (Figure 3.8A) and August to November 2009 (Figure 3.8B) where a relatively higher percentage coverage of oysters was also observed. Tiles from AYW shallow and AMW deep water had significantly higher coverage of oysters than those from the other two sites in AMW (Two-way ANOVA, $p<0.01$;Tamhane's test, $p<0.05$). This difference, however, was not clearly marked between the face up and face down tiles in any specific sites (Tamhane's test, $p>0.05$).

The coverage of bryozoans varied significantly in different sites, as well as between face up and face down tiles over time (Two-way ANOVA, $p<0.01$; Figure 3.8). Tiles from August to November 2009 (Figure 3.8B) and the one year tiles (Figure 3.8G) were found to have significantly higher % coverage of bryozoans than tiles from Nov 2009 to Jan 2010 (Figure 3.8C), but not with the rest (Two-way ANOVA, $p<0.01$; Tamhane's test, $p<0.05$). The coverage of bryozoans was significantly higher in

AYW shallow and deep than in the other two sites in AMW, with face down tiles having significantly higher coverage than the face up tiles from all sites throughout the study period (Tamhane's test, $p<0.05$).

Tube worms contributed the least to the total % coverage of fouling organisms on tiles throughout the year (Figure 3.8). However, their abundance still varied significantly in different sites, as well as between face up and face down tiles over time (Two-way ANOVA, $p<0.05$). Tiles from January to April 2010 (Figure 3.8D) had significantly higher % coverage of tubeworms than the rest of the tiles collected (Two-way ANOVA, $p<0.05$; Tamhane's test, $p<0.05$). However, no specific sites at any one time stood out to be distinctly different from the rest (Tamhane's test, $p>0.05$). Overall, the coverage of tubeworms was significantly higher in face down tiles than in face up tiles for all the sites except in AYW shallow (Two-way ANOVA, $p<0.05$; Tamhane's test, $p<0.05$).

3.3.2 Coral recruitment on concrete blocks

After 1.5 years, the number of recruits recorded on the horizontal surface of concrete blocks ranged from 1 to 16 in AYW, and 1 to 2 in AMW (Figure 3.10). Overall, an average of 2.29 recruits per block was recorded over the 1.5 years of study period, or

an equivalent of 16.61 spats m^{-2}. Of the total number of 55 recruits observed, two belonged to Family Poritidae and 53 belonged to Family Faviidae. Among these Faviids, 50 were *Oulastrea crispata*. Comparing between depths, 3/55 (5.45%) recruits settled in deeper water, while 52/55 (94.55%) recruits settled in shallow water. For all sites combined, maximum recruitment 49/55 (89.09%) occurred in AYW shallow water, followed by 3/55 (5.45%) in AMW shallow, 2/55 (3.64%) in AYW deep and 1/55 (1.82%) in AMW deep water.

Images of recruits were taken using a Canon® G10 digital camera and recruit sizes were measured using a computer image analysis program, Image-Pro® Plus 6.0. The minimum diameter and surface area of *O. crispata* recruits recorded were 0.75 mm and 0.37 mm^2 respectively, while the maximum were 16.62 mm and 181.11 mm^2 respectively. *Oulastrea crispata* recruits can be as small as having only one polyp while a maximum of 27 polyps were recorded from a Poritid recruit. Of the total number of 55 recruits observed, 3/55 (5.45%) were being partly overgrown by turf algae while the rest (52/55 or 94.55%) were undamaged.

3.3.3 Environmental parameters

An annual mean (± SD) sea water temperature of 24.84 ± 4.45℃ in shallow water

and 24.61 ± 4.30 ℃ in deep water were recorded for TPCMP, with a minimum of 15.7℃ recorded in 19 Feb, 2010 and a maximum of 31.9℃ recorded in 17 August, 2010 (Figure 3.11). June to October was the warmest period of the year (28.48 ± 1.60℃), in contrast to December to April (18.93 ± 1.36℃). Thermocline was observed in both AYW and AMW in July and August, with the average sea water temperature in deep water (28.71 ± 1.82℃) being slightly lower than that in shallow water (29.53 ± 1.22℃).

An annual mean (± SD) sedimentation rate of 48.8 ± 7.2 mg cm^{-2} d^{-1} was recorded from the study sites, with a maximum of 61.7 ± 15.3 mg cm^{-2} d^{-1} recorded from AMW shallow, followed by 49.5 ± 2.5 mg cm^{-2} d^{-1} from AMW deep, 45.1 ± 6.6 mg cm^{-2} d^{-1} from AYW shallow and a minimum of 38.9 ± 4.3 mg cm^{-2} d^{-1} from AYW Deep (Figure 3.12). The exceptionally high sedimentation rates (up to 226 ± 111.3 mg cm^{-2} d^{-1} from AMW shallow water) in August 2010 were due to the influence of two tropical cyclones, Conson and Chanthu (Hong Kong Observatory, 2010b). AMW shallow also recorded a higher rate of sedimentation in a few other months (e.g. April and November, 2010).

3.3.4 Post-settlement survival of artificially seeded coral recruits of *Platygyra acuta*

The mean number of surviving artificially seeded coral recruits dropped significantly over the first two samplings (half a months and 1.5 months) for both face up and face down tiles from the four sites (Figure 3.13). The total overall post-settlement survivorship was <30% and <1% respectively. There was no significant difference in the recruit survivorship among sites (Two-way ANOVA, Sites: $F=0.537$, $df=3$, $p=0.659$), but the drop in recruit survivorship was significant over time (Two-way ANOVA: Time: $F=289.539$, $df=3$, $p<0.001$) (Figure 3.13C). The interaction between site and time was not significant (Two-way ANOVA: Site*Time: $F=0.549$, $df=9$, $p=0.833$). After the first sampling (half a month), the overall mean (± SD) survivorship of the seeded recruits was highest in AYW shallow (33.18 ± 29.05%), followed by AMW deep (20.89 ± 35.29), AYW deep (18.92 ± 29.90) and AMW shallow (13.57 ± 34.88). There were no significant differences in the post-settlement survivorship of the recruits between the face up and face down tiles among sites (Two-way ANOVA, Site: $F=0.570$, $df=1$, $p=0.455$; Facing: $F=0.700$, $df=5$, $p=0.627$, Figure 3.13A, B). At the end of the experiment on Oct 5, no recruits on the heavily silted face up tiles survived, while 0.78 ± 1.74% survived on face down tiles from AYW deep (Figure 3.13A, B).

3.3.5 Effects of gastropod exclusion on post-settlement survival of coral recruits

Gastropod exclusion did not appear to exert a significant effect on the survival of coral recruits. There were no significant differences in the percentage of surviving recruits between treatment and sites at each sampling date (Two-way ANOVA, $p>0.05$) (Table 3.1, Figure 3.14). In AMW shallow, the mean percentage of surviving recruits in gastropod exclusion cages (44.8 ± 30.4%) was initially lower than that in the other control treatments in the first sampling (half a month) (Figure 3.14A), although the difference was not statistically significant (One-way ANOVA, $p>0.05$). Thereafter, recruit mortality increased rapidly in the subsequent months, especially in the cage controls, but that of the recruits in the gastropod exclusion cages was not high. At the end of the experiment on Oct 7, the percentage of surviving recruits in the gastropod exclusion cages was the highest (18.2 ± 13.5%) among all treatments, but this difference was not statistically significant with those in the controls (One way ANOVA, $p>0.05$). Overall, there were no significant differences in the percentage of surviving recruits among treatments (Two-way ANOVA, Treatments: $F=0.935$, $df=3$, $p=0.435$), although the drop in the percentage of surviving recruits was significant over time (Two-way ANOVA, Time: $F=115.844$, $df=3$, $p<0.001$). No interaction between treatments and time was detected (Two-way ANOVA,

Treatment*Time: F=0.949, df=9, p=0.498).

In AMW deep water, the mean (± SD) percentage of surviving recruits in gastropod exclusion cages (80.6 ± 11.4 %) was initially higher than that in all the controls (e.g. control and cage controls) in the first sampling (half a month), but this difference was not statistically significant among all treatments (One way ANOVA, $p>0.05$) (Figure 3.14B). However, this survisorship dropped rapidly and towards the end of the experiment on Oct 7, >95% of the recruits died. Overall, there were no significant differences in the percentage of surviving recruits among treatments (Two way ANOVA, Treatments: F=1.438, df=3, p=0.250) and the drop was significant over time (Two-way ANOVA, Time: F=207.896, df=3, $p<0.001$). No significant interaction between treatment and time was also detected (Two-way ANOVA; Treatment*Time: F=0.957, df=9, p=0.492).

3.4 Discussion

3.4.1 Coral settlement

Low recruitment rate on settlement tiles, as well as high mortality of pre-seeded recruits as revealed in this present study, indicates the difficult conditions these recruits need to face to survive in the study sites. Settlement tiles were monitored

closely in the field on a weekly basis during and immediately after the spawning seasons (from April to October) and bi-weekly during winter (November to March) using the fluorescence census technique. Any newly settled recruits on the settlement tiles should have been detected. Additionally, these tiles were brought back to the laboratory for closer examination under the microscope but no skeletal remnant of any coral recruit was observed. This strongly suggests that settlement on the settlement tiles itself was very low.

3.4.1.1 Low settlement rate of corals

Both short and long term monitoring on the settlement tiles showed similar coral recruitment of 0.94 recruits m^{-2} throughout the study period. On the other hand, recruitment density was higher on concrete blocks at 16.61 recruits m^{-2}. The latter, however, was contributed mainly (90.9%) by the recruits of *Oulastrea crispata*. This Faviid is a pioneering species that easily colonizes newly opened space. It generally prefers flat rather than vertical surfaces and may prefer the concrete blocks more than the settlement tiles, the only surviving recruit on the settlement tiles in the present study was likely to be *O. crispata* as well. This species has been reported to be both broadcast spawner and planulae brooder in Japan and Hong Kong (Nakano and Yamazato, 1992; Lam, 2000). Brooding corals are generally considered to have

lower dispersal distances (Harrison and Wallace, 1990; Harii and Kayanna, 2003). With this species being very dominant in shallow water in the study sites, its brooding characteristics could explain its exceptionally high contribution (48/50 or 96%) to the number of recruits in the concrete blocks. On the other hand, 2.5 years monitoring of the reproductive biology of this species from the present study sites earlier did not find brooding larvae in this species (Lin, 2003). Instead, gametogenesis was found to be asynchronous and potential spawning occurring several times in a year. This latter could also contribute to high recruitment, with larvae being available all year round.

Overall, the data on settlement tiles and concrete blocks revealed extremely low recruitment success in the present study sites, when compared to that reported in other tropical areas: up to 300- 4000 recruits m^{-2} $year^{-1}$ in the Great Barrier Reef (Glassom *et al.*, 2004), 101-908 recruits m^{-2} $year^{-1}$ in Kenya (Mangubhai *et al.*, 2007), and up to 32.5 recruits m^{-2} $year^{-1}$ in Southern Taiwan (Kuo and Soong, 2010). The situation is more similar to Singapore with only 1.4 ± 1.0 to 20 ± 14.7 recruits m^{-2} $year^{-1}$ recorded (Dikou and Woesik, 2006).

3.4.1.2 Possible causes of low coral settlement

Low recruitment success may be due to failure in any of the pre-settlement and post-settlement processes, including the availability of competent larvae, the probability that the larvae will settle, and the survival of new recruits after settlement (Keough and Downes, 1982; Connell, 1985; Abelson *et al.*, 2005). In the study sites, annual massive spawning of scleractinian corals involving multi-species as well as multiple colonies of the same species have been observed regularly in summer, from May to July, followed by some smaller scales of spawning (refer to Chapter Two for details). Availability of gametes is thus unlikely to be a problem. However, how many of these gametes could fertilize, survive through planktonic stages and become competent, or whether competent larvae are available for settlement remains unknown. The monsoon season in Hong Kong starts in May or June and major coral spawning events could coincide with heavy rainfall (Hong Kong Observatory, 2009b, 2010b). Decreased salinity from high precipitation or from land run of, increased in nutrient loadings and in the level of suspended particulates or sedimentation could have severe effects in reducing fertilization successes, altering larval behavior, reducing larval survival and settlement (Sammarco, 1980; 1991; Babcock and Davies, 1991; Gilmour, 1999; Vermeij *et al.*, 2006).

Dispersal ability of larvae varies between brooding and broadcasting corals. Previous studies have indicated that larvae released from brooding corals are able to settle immediately. Maximum rate of settlement occurs within a few days after planulae release, or the larvae can also survive and settle up to 100 days (Richmond, 1987; Harrison and Wallace, 1990). For broadcasting corals, planulae larvae require a minimum planktonic developmental period of 2.5 to 6 days to become competent to settle (Babcock and Heyward, 1986; Harrison and Wallace, 1990; Miller and Mundy, 2003). Although maximum competency can be up to several weeks, maximum settlement rates of coral larvae usually lie between 10 to 32 days after spawning (Wilson and Harrison, 1998). Hence, the larvae of brooding corals are generally considered to have lower dispersal distances than broadcasting corals and self-seeding is more likely to occur in brooding than in broadcasting corals (Harrison and Wallace, 1990; Harii and Kayanna, 2003). The most dominant corals in the study sites, as well as in Hong Kong, like *Platygyra* spp., *Favia* spp. *Favites* spp., *Porites* spp., *Acropora* spp. and *Pavona* spp., are all known broadcasters. The length of their larval developmental period in Hong Kong, however, is largely unknown. Nonetheless, given that some period of at least a few days would be needed for larval development, the time is sufficient for them to be drifted away from their spawning grounds. The data obtained in this study, from *in situ* fluorescence census and

microscopic examination of settlement tiles, showed that the very few coral recruits settled only from August to November, a few months after the major local spawning period in May to June. No natural recruits were recorded from May to July on the settlement tiles. This is possible only if the larvae developed from the major mass spawning events had a long competency ability (up to 60-100 days) and could remain in the water before settlement (Richmond, 1987; Wilson and Harrison, 1998, Nozawa and Harrison, 2000), or that these recruits were simply from the small scale spawning after July.

On the other hand, TPCMP, where our study sites are located, is a small, open island with no major enclosed bays. It is not likely to have a high ability to trap nor retain coral larvae. This is evidenced from the lack of retention of slicks along its shore even after a major spawning event overnight. Eggs and larvae have the high probability of being dispersed away from the spawning sites by tidal and wind-driven current before they become competent. Thus coral larvae that eventually settled on the settlement tiles or concrete blocks, except for those of *O. crispata*, may not be from the local coral communities (i.e. from non-localized sources). The lack of any coral recruits that settled on the settlement tiles in the second year (2010) strongly suggests that the arrival of larvae could be very sporadic and highly variable between

years. It is also possible that some of these source sites have been disturbed in recent years, such that they could no longer supply sufficient amount of competent larvae for successful recruitment in the present study sites. Non-localized sources of coral larvae from adjacent coral communities had been suggested in other studies (Grigg *et al.*, 1981; Veron, 1985; Wallace, 1985 Nozawa and Harrison, 2000). A future study of the hydrodynamics of the coastal current patterns in Hong Kong or around TPCMP should be able to confirm this speculation.

A low probability of larval settlement could also result from low abundance of settlement cues, as appropriate chemical cues from biofilms or crustose coralline algae are known to be critical for the settlement of coral larvae (Morse *et al.*, 1988; Heyward and Negri, 1999; Negri *et al.*, 2001; Webster *et al.*, 2003). However, all settlement tiles used in the present study were pre-conditioned *in situ* for at least 6 weeks prior to their deployment in the experiments. Biofilms as well as crustose coralline algae were evidently growing on them. Low recruitment was similarly recorded on tiles exposed for 3 lunar months or for the whole year, suggesting that the lack of settlement cues may not have contributed to the low settlement of larvae.

3.4.2 Post-settlement survival of coral recruits

Even if recruitment was successful locally after the major spawning event in May to June, evidences from the present study indicated that the post settlement survivorship of coral recruits would still be very low. Almost none of the pre-seeded coral recruits of *P. acuta* survived after four months in the field. A post-settlement mortality of up to 78.36% was already recorded within half a month and 99.9% after 3.5 months of deployment of the pre-seeded ceramic tiles *in situ*.

3.4.2.1 Effect of high sedimentation rate

High sedimentation rate may possibly be one of the reasons for this recruitment failure by reducing the survival rate of early life stages of recruits. Previous study (Rogers, 1990) had suggested mean sedimentation rates of > 10 mg cm^{-2} d^{-1} to be "high" for corals. Our data recorded a much higher mean sedimentation rate of 48.8 ± 7.2 mg cm^{-2} d^{-1} and a maximum of 226 ± 111.3 mg cm^{-2} d^{-1} during monsoon season. In addition to re-suspension of sediments from tropical storms during monsoon season, large scale land reclamation or development associated with port, industrial, housing, resort and hotel development in surrounding areas of TPCMP in recent years has increased the sedimentation rate significantly. Sedimentation rates in AMW and AYW recorded 10 years before (Wong, 2001) were as low as 0.87mg cm^{-2}

d^{-1} in AMW in July, 1999, with the highest of 275.9 mg cm^{-2} d^{-1} recorded in AMW in June, 1999 during monsoon season. These data reveal that the sedimentation rate in TPCMP has increased significantly over the past 10 years. Sedimentation stresses together with the associated turbidity are known to negatively affect coral settlement rates and reduce recruit survival (Rogers *et al.*, 1984; Sammarco, 1980; 1991; Hudgson, 1990; Tomascik, 1991; Fabricius, 2005; 2011). Therefore, recruits in the present study sites are undoubtedly under tremendous pressures from this high sedimentation impact. Different coral species have different tolerances to sedimentation, and high sedimentation stress could lead to a reduction in biodiversity (Fabricius, 2005). This is also reflected in the present study wherein majority (82.3%) of the recruits on the concrete blocks were *O. crispata*, the coral species known to be resistant to adverse environmental conditions, like heavy siltation (Nakano and Yamazato, 1992) and cold temperature (Yajima *et al.*, 1986).

3.4.2.2 Competition and predation by other marine organisms

Competition for settlement space is clearly very keen in the study sites. The settlement tiles were quickly fouled, with up to an average of 89.8 ± 11.66% and 79.51 ± 15.15% respectively of the surface of the short-term (November 2009 to January 2010) and long-term (1 year) settlement tiles being covered with fouling

organisms. With the significantly higher coverage of barnacles (November to January), bivalves (May to November) and bryozoans (May to November) following spawning seasons, even if the larvae did settle, subsequent growth of these fast growing fouling organisms could easily overwhelm the recruits, causing early post-settlement mortality (Chui and Ang 2010). Competitive interactions of sessile organisms are well known to inhibit settlement and reduce growth and / or survival of new coral recruits (Hunte and Wittenberg, 1992; Tomascik, 1991; Avigdor *et al.*, 2005; Chadwick and Morrow, 2011). High coverage of fouling organisms in settlement tiles appears typical of polluted environment. In North Beach, a major eutrophication source in Eilat, Israel, up to 100% coverage of fouling species were observed during short-term (5 months) deployment of recruitment tiles (Avigdor *et. al*, 2005). Near a fish farm in Bolinao, Philippines, up to 80% coverage of barnacles on settlement tiles were observed after 81 days of deployment (Villanueva *et al.*, 2006). The high rate of fouling on settlement tiles found in the present study suggests a deterioration of water quality of the study sites. This would have a serious implication not only on coral recruitment success, but also on the health of the whole coral communities as well.

Macroalgae are also known to adversely affect the survival of corals, including

growth and recruitment, by outcompeting coral recruits directly though shielding off of sunlight and/or by sediment-trapping (Walker and Ormond, 1982; Tomascik, 1991, Lirman, 2001; McCook *et al.*, 2001; Fabricius, 2011). Turf algae were found on tiles throughout the year, causing mortality of some coral recruits that settled on these tiles. Furthermore, the growth of the macroalgae *Colpomenia sinuosa* (field observation) from March to April covered up to 100% of the surface of face up tiles in shallow water of both sites. This would have some effects on recruit survival as well.

Predation by gastropods could also affect the post-settlement survival of new coral recruits. Corallivorous gastropods *Drupella rugosa* and *Cronia margariticola*, were observed throughout the year on settlement tiles. Population outbreaks of *Drupella*, particularly in Western Australia and Japan, caused drastic disturbance to coral reefs by reducing coral cover. The destructive potential of high density of *Drupella* on coral reefs is comparable to that of the crown-of-thorns starfish *Acanthaster planci* (Turner, 1994). However, results of the predator exclusion experiments in the present study suggest that the impact of predation itself may not be as great as that of competition with fouling organisms. In fully enclosed cages, the exclusion of gastropod predators actually led to an increase in the cover of oysters as oysters were

also released from predator pressures. This increase likely caused the high mortality of coral recruits even on tiles that were enclosed in full cages.

3.5 Summary

Overall, the data from settlement tiles and concrete blocks revealed the extremely low natural recruitment success of corals in the study sites. This is mainly due to low settlement rate of the coral larvae, which may have resulted from a lack of competent larvae retained within the sites. Although mass spawning of corals were observed, much of the larvae developed from the spawning events may have been drifted away from the spawning sites. TPCMP itself may not be effective in trapping larvae from other sites, or the sources of larvae that regularly come to TPCMP may have been disturbed and could no longer supply enough competent larvae that would eventually settle around the island. On the other hand, results from experiment examining the survival of coral recruits of *Platygyra acuta* pre-seeded on ceramic tiles showed that these coral recruits experienced very high mortality following settlement. Average mortality of 78.36% was observed within half a month of tile deployment *in situ*, < 1% survived through the first four months of deployment. Low post-settlement survival is suggested to have resulted from high sedimentation, intense competition for space with other fouling organisms and predation effects. Predator exclusion

experiments indicated that reducing predation effect may indirectly increase the growth of fouling organisms like oysters as they were also released from predation pressure. Thus no increase in the survival rates of the coral recruits were detected under the "predator free" condition. Competition with fouling organisms for space may be the most important factor that contributed to high post-settlement mortality of corals.

This study provided the baseline information critical to the understanding of coral recruitment processes in Hong Kong coral communities, typified by those in TPCMP. However, as settlement rate of corals has been shown to be low and to fluctuate between years and sites, as reported elsewhere outside Hong Kong (Wallace, 1985; Hughes *et al.*, 1999), a longer term monitoring of coral recruitment in a wider spatial scale is needed to provide a better picture of the coral recruitment status in Hong Kong. The importance and significance of this understanding cannot be underestimated. If recruitment failure is consistently repeated over years and over different sites, it means that it may take a long time for Hong Kong coral communities to recover from any natural or human induced disturbances. Or that a permanent phase shift may occur and coral communities in Hong Kong may be gone forever.

Long term monitoring study has indicated that Hong Kong coral communities are subjected to repeated physical disturbances (typhoons or severe tropical storms) and appeared to be relatively stable and resistant. However, they may have low resilience towards more sever or chronic disturbances in the future (Tam and Ang, 2008). A case in point is the increase in the number of fouling organisms that competed for space with coral recruits. This increase in the number of fouling organisms is an indication of the deteriorating quality of the seawater environment in Hong Kong that will not favor natural recruitment success of corals. While measures should be put in place to improve Hong Kong marine environment, other strategies may also be developed to enhance coral recruitment success. Coral nursery may be developed to grow coral recruits that could eventually be transplanted to denuded sites. Other protective measures should of course be put in place to help restoring the coral communities. The increasing threats faced by Hong Kong corals and coral communities are not unique to Hong Kong but are being documented in many other places as well. Development of future effective management strategies for the conservation and restoration of coral communities in Hong Kong should find applications in other comparable subtropical areas in southern China as well as in the Indo-west Pacific.

Table 3.1 Mean (± SD) number and proportion (%) of surviving recruits under different treatments in the gastropod exclusion experiment over time. Percentage survivorship was calculated based on the initial number of recruits.

Treatments	Sampling Dates							
	17 June (Starting)		7 July		22 August		7 October	
	Number	%	Number	%	Number	%	Number	%
A. AMW Shallow								
Control	59 ± 26.46	100	36.67 ± 16.80	63.8 ± 19.9	12 ± 8.54	20.0 ± 10.5	6.33 ± 4.73	11.3 ± 9.2
Top-half cage	82.67 ± 40.20	100	48.67 ± 36.94	57.8 ± 24.5	2.67 ± 3.79	2.6 ± 2.8	1.67 ± 2.89	1.3 ± 2.3
Side-half cage	47 ± 26.23	100	29.33 ± 17.21	60.7 ± 19.2	2.67 ± 3.06	5.6 ± 4.8	1 ± 1	2.3 ± 2.2
Full cage	99.67 ± 35.23	100	51.67 ± 48.95	44.8 ± 30.4	27.33 ± 26.03	23.6 ± 18.7	20.67 ± 18.50	18.2 ± 13.5
B. AMW Deep								
Control	68.67 ± 26.35	100	38.33 ± 8.62	64.3 ± 34.2	1.67 ± 2.08	3.4 ± 4.5	0.33 ± 0.58	0.7 ± 1.2
Top-half cage	223.33 ± 58.48	100	142 ± 52.46	62.4 ± 11.0	16 ± 3.61	7.8 ± 3.4	2.33 ± 3.21	1.3 ± 1.9
Side-half cage	204.67 ± 273.07	100	109 ± 136.63	60.1 ± 7.7	34 ± 42.51	24.1 ± 19.3	6.33 ± 8.39	4.1 ± 3.2
Full cage	229 ± 89.11	100	189.33 ± 97.70	80.6 ± 11.4	36.33 ± 14.01	4.1 ± 6.3	9.33 ± 6.66	4.0 ± 3.2

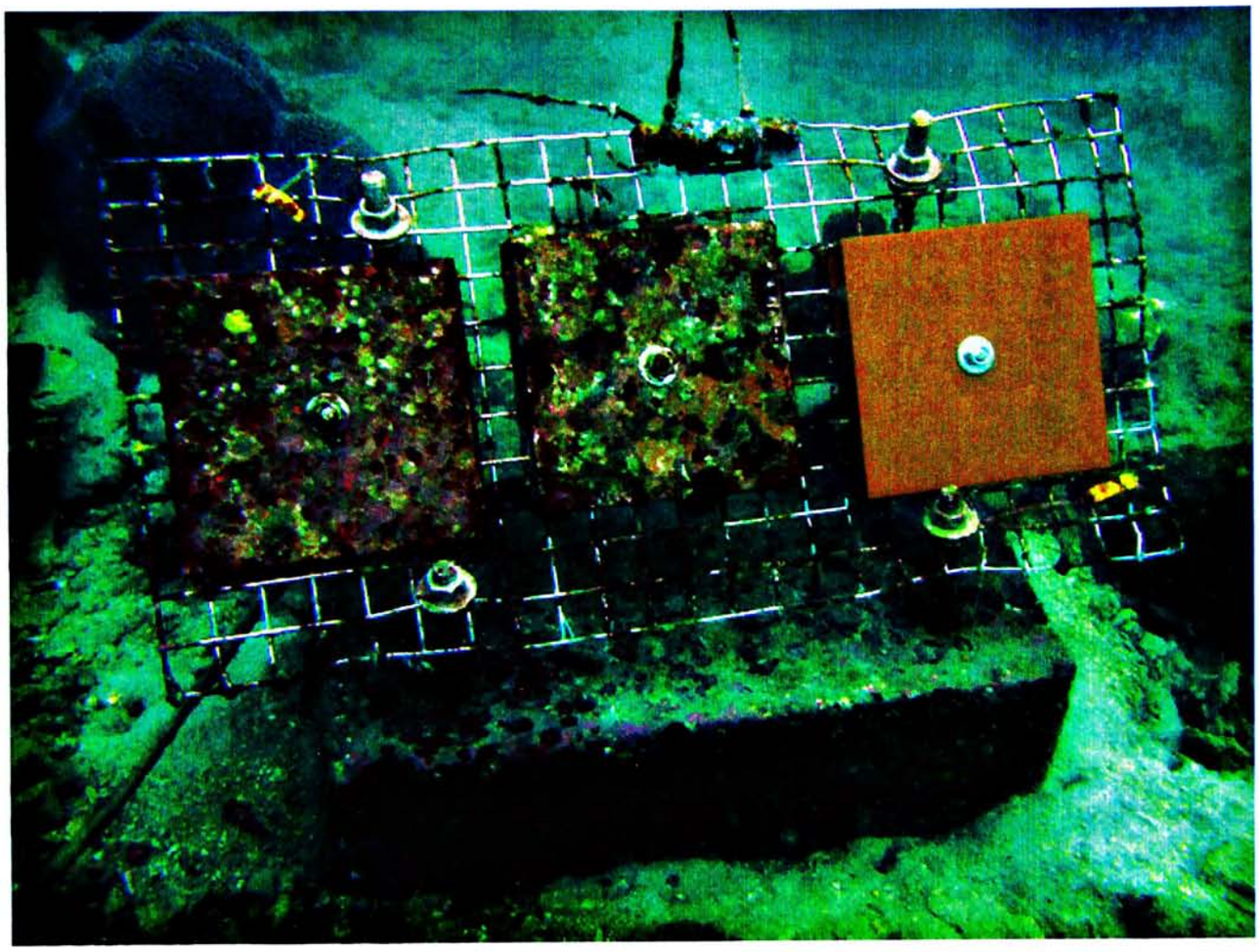


Figure 3.1 Experimental setup, with terracotta tiles mounted at 45 degrees on stainless steel mesh. The whole setup is supported by a concrete block. The Minilog temperature logger can be seen deployed on the top corner of the set-up.

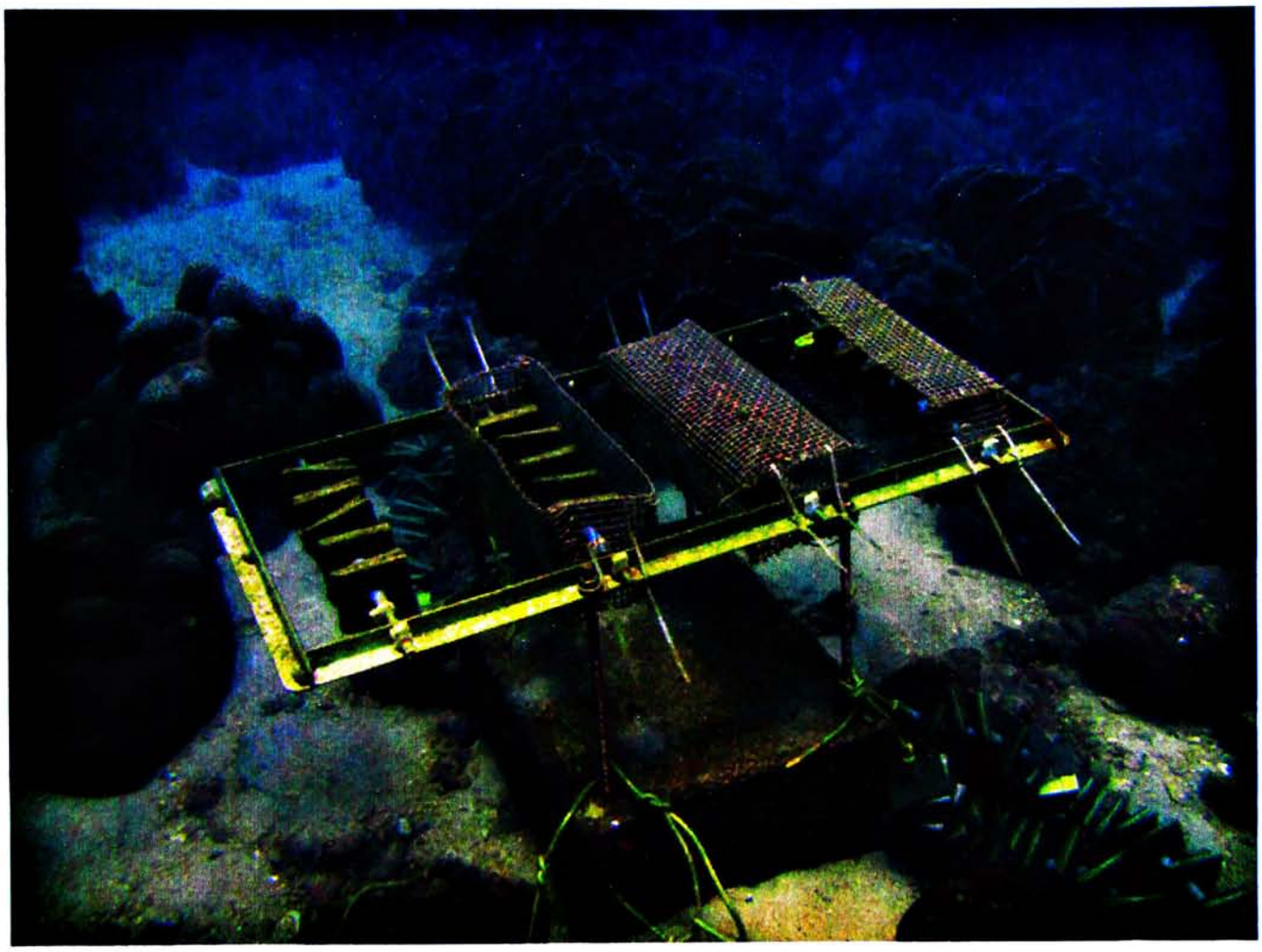


Figure 3.2 Setup for cage experiment against predation effect. Four tile rods, each with five pairs of ceramic tiles, are mounted on concrete block, As an example, the tile rods shown in the picture are for the following treatments, from left: control with no cage, side-half cage (cage control), full cage and top-half cage (cage control).

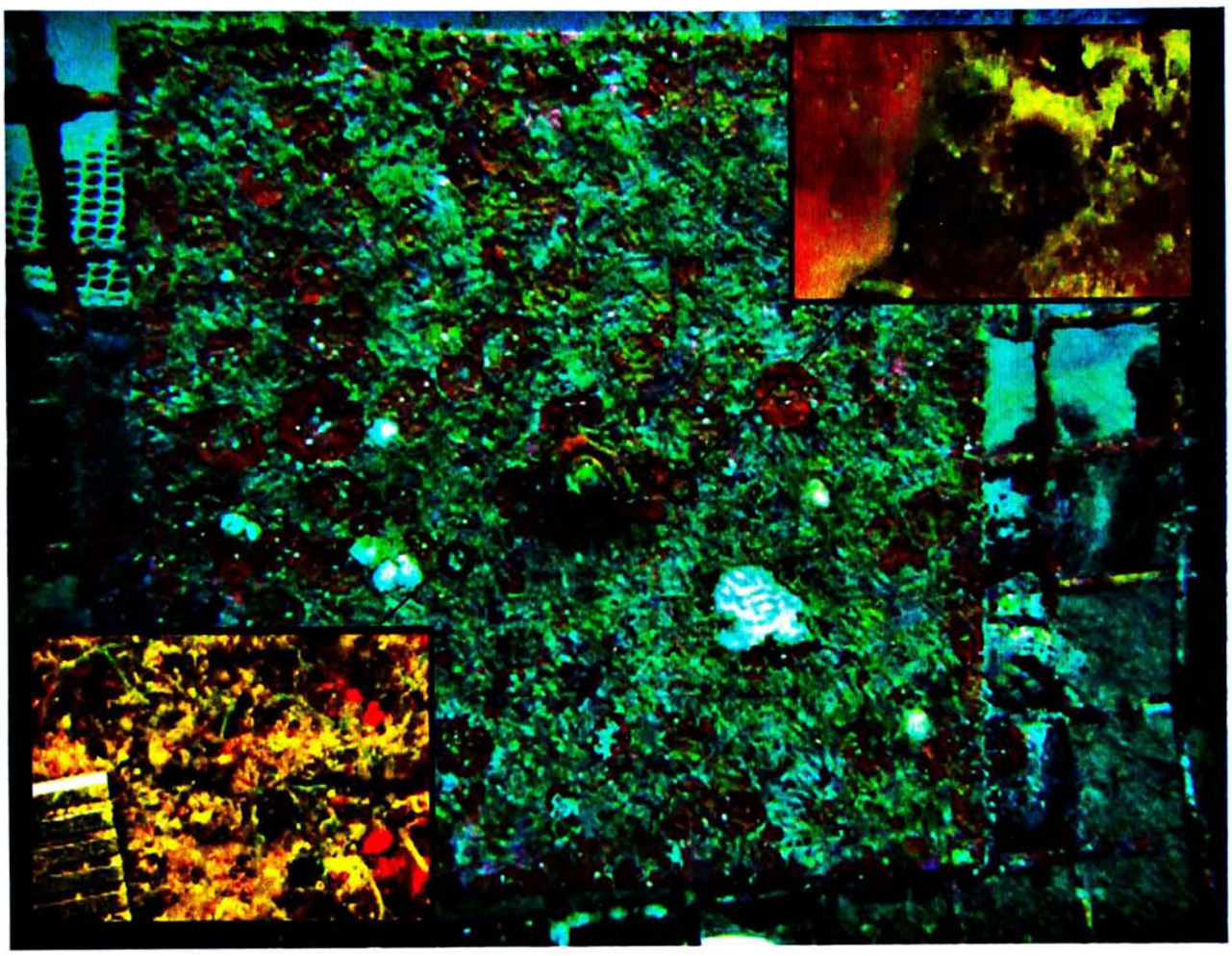


Figure 3.3 Settlement tile used in short-term (3 lunar months) monitoring for coral recruits. This tile was placed in a face up position and two coral recruits that settled are indicated by circles.

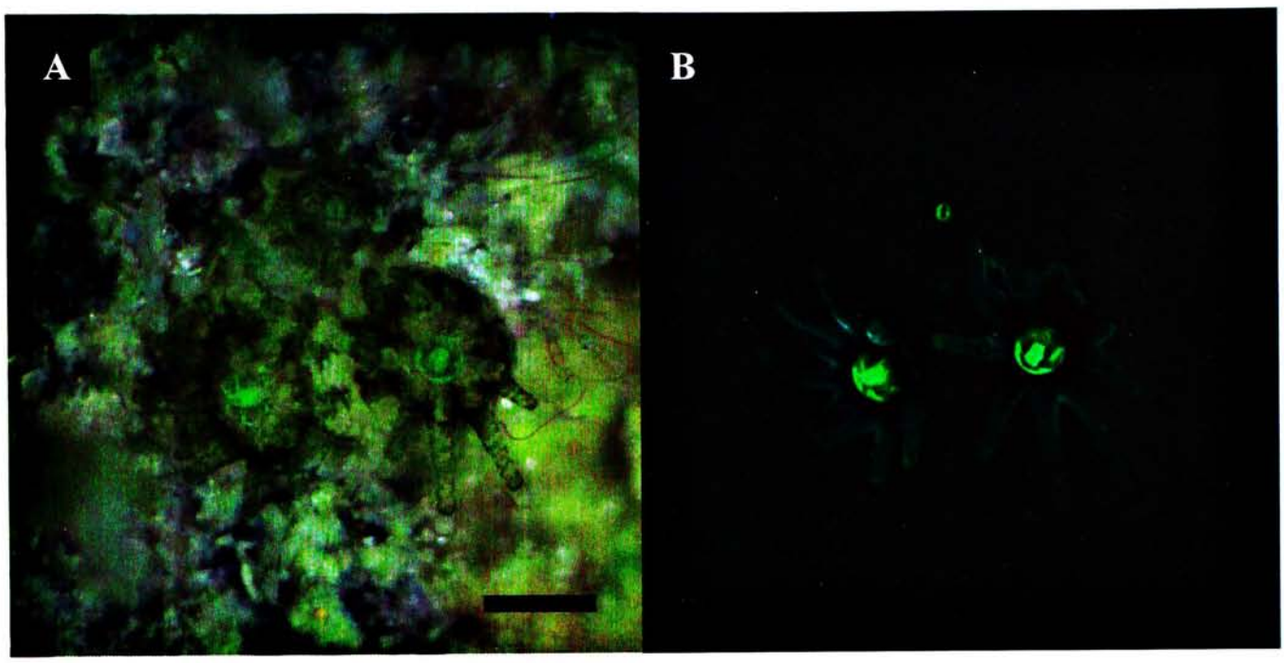


Figure 3.4 Photograph showing coral recruits (Poritidae) under the dissecting microscope (**A**) and fluorescence image of the same (**B**) on settlement tile deployed in TPCMP. Scale bar =0.5mm

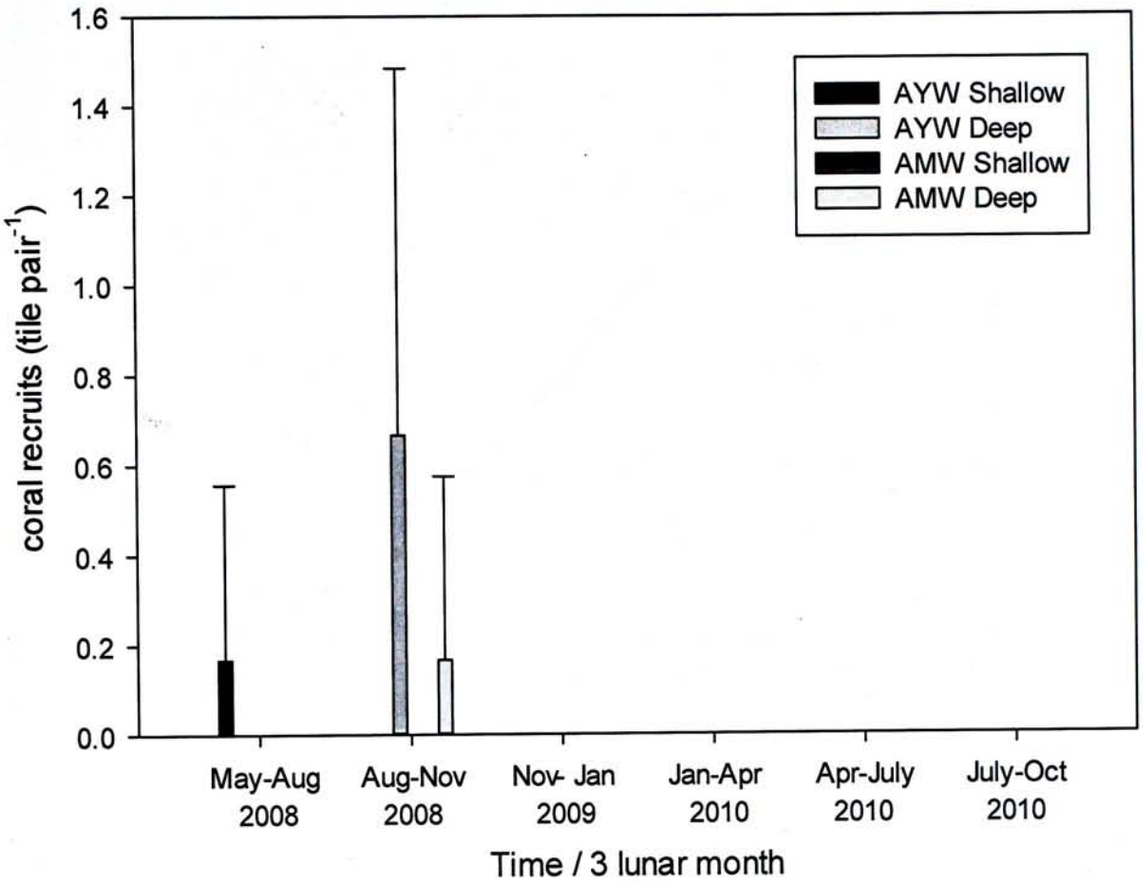


Figure 3.5 Mean (± SD) density of coral recruits per pairs of terracotta tiles recorded throughout the monitoring periods in the four study sites in TPCMP from May 2008 to October 2009.

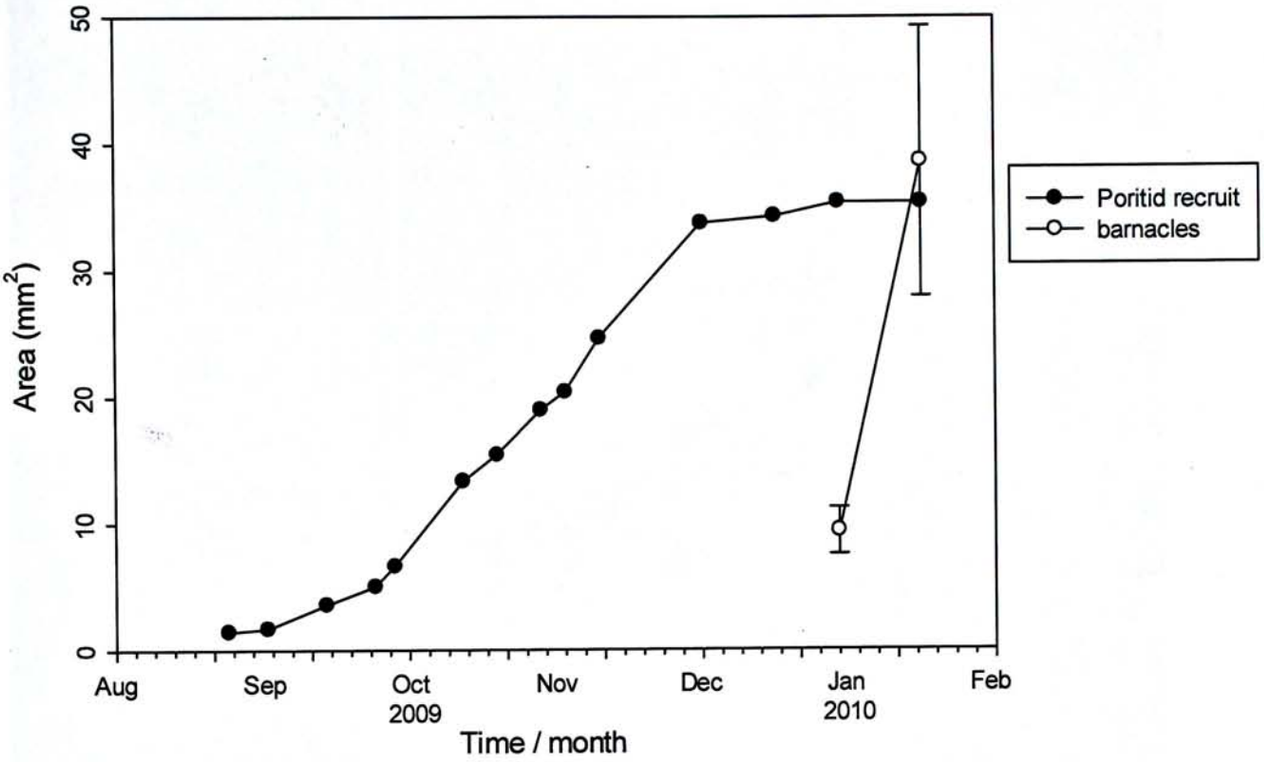


Figure 3.6 Growth of Poritid recruit and mean (± SD) diameter of barnacles (n=10) on terracotta tiles deployed in AYW shallow water. Barnacle outbreak occurred in November 2009 to January 2010 and rapid growth of barnacles subsequently overgrew the recruit.

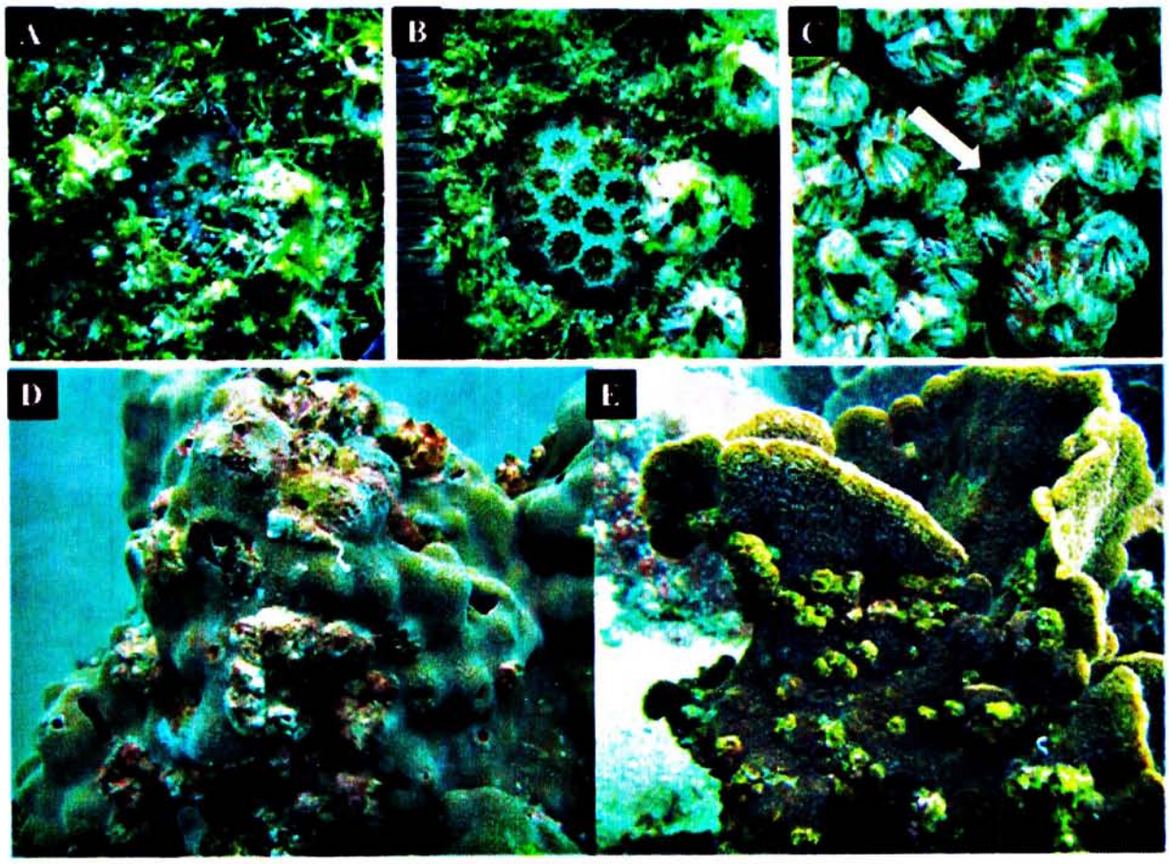


Figure 3.7 (**A**) Coral recruit (Poritidae) on settlement plate covered with sediments (photo taken on 22 Dec, 2009); (**B**) Same coral recruit with sediments removed, showing the recruitment of barnacles around it; (**C**) Same coral recruit (arrow) being overwhelmed by overgrowth of barnacles on 8 Jan, 2010; (**D**) and (**E**) Massive recruitment of barnacles that occupied open spaces on the surface of various coral colonies.

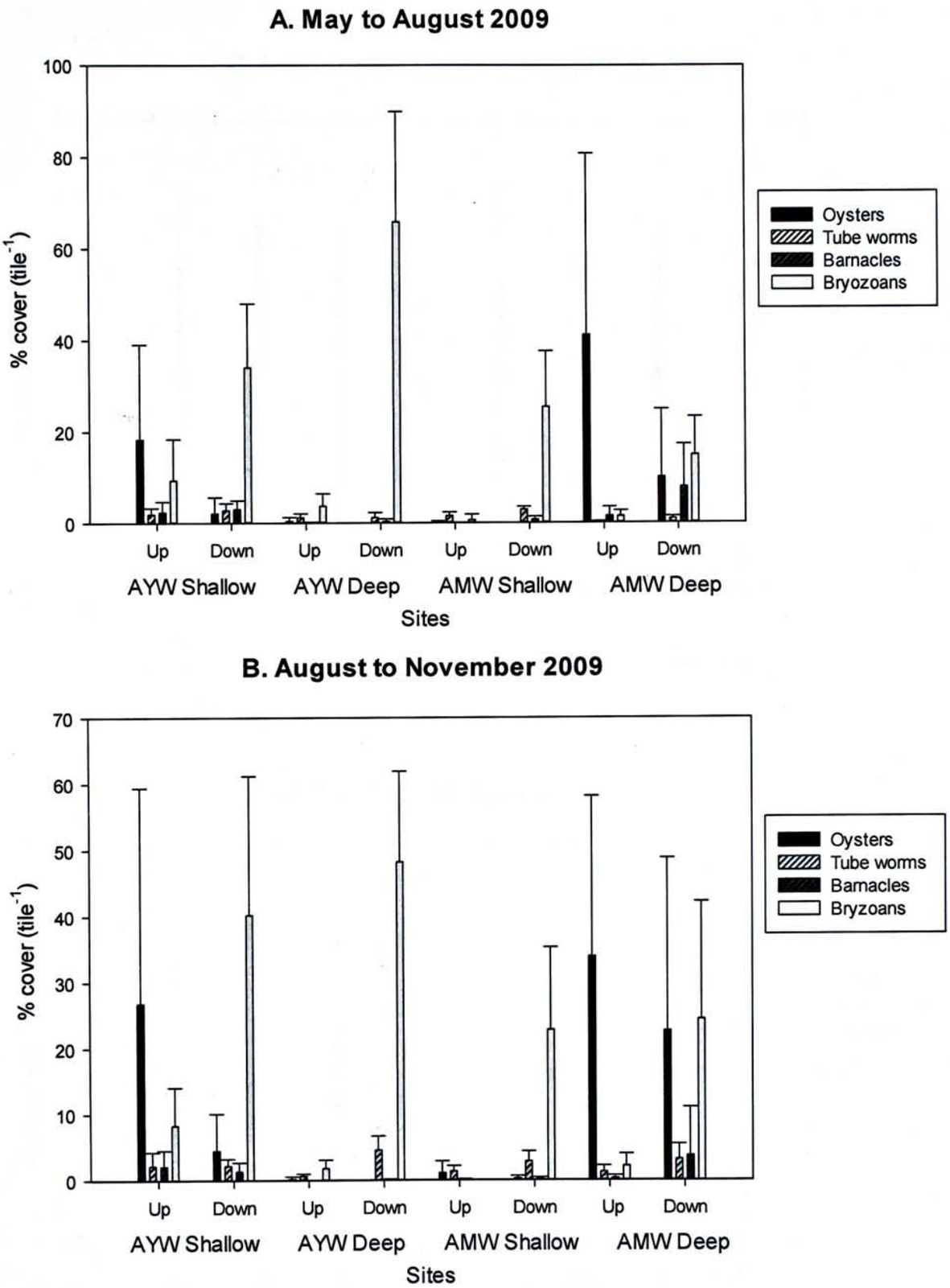
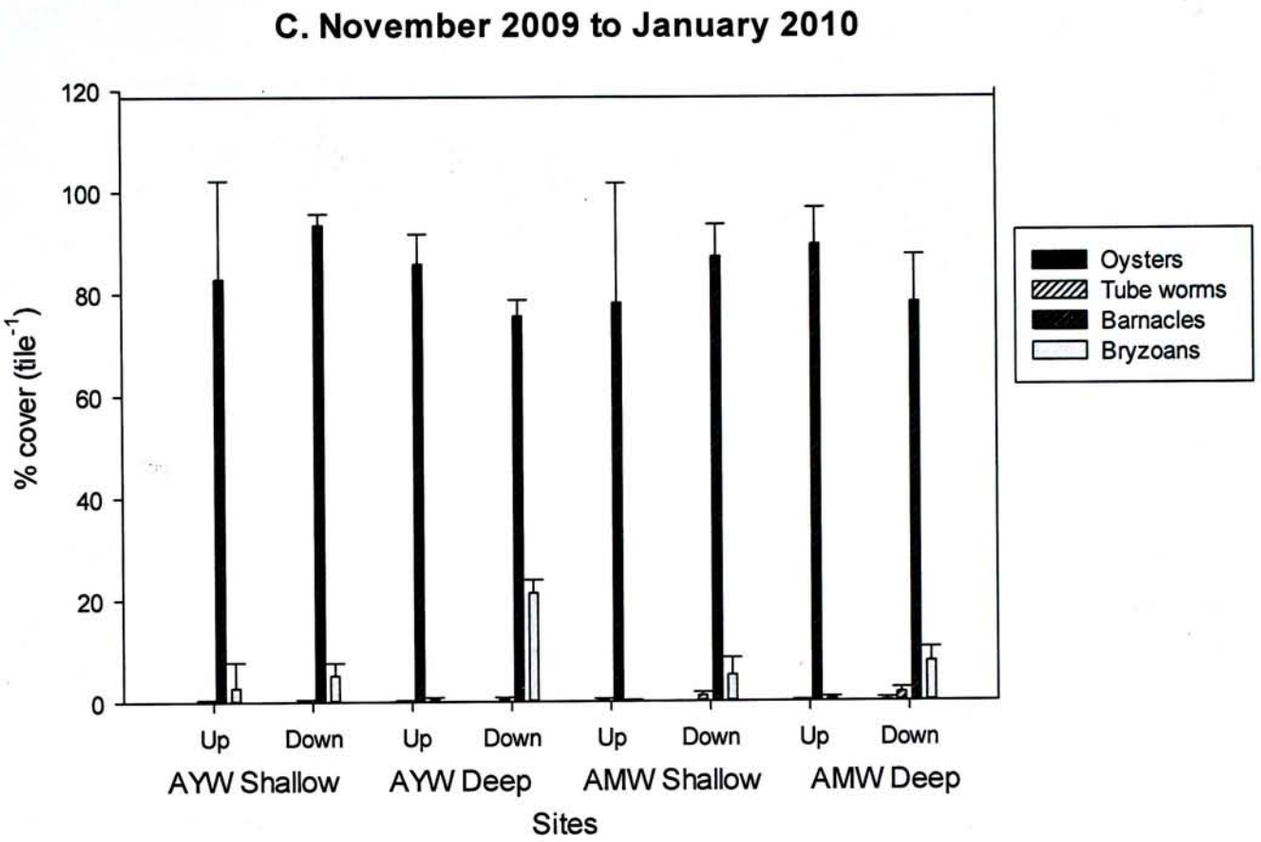


Figure 3.8 Spatial and temporal variation in the mean (± SD) percent cover of major fouling organisms on settlement tiles deployed in different sites in TPCMP. The tiles were deployed in pairs for three lunar months each time with one tile facing up and one facing down.



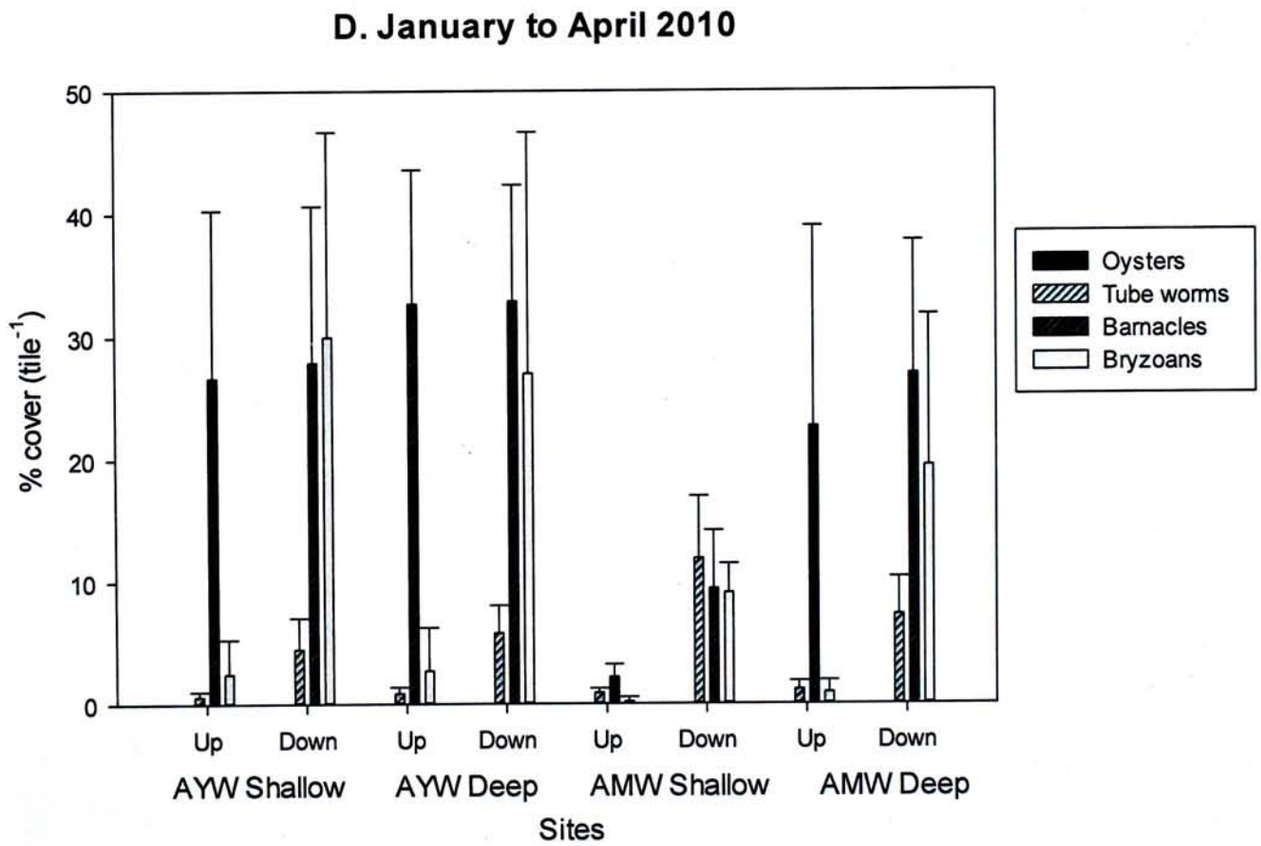
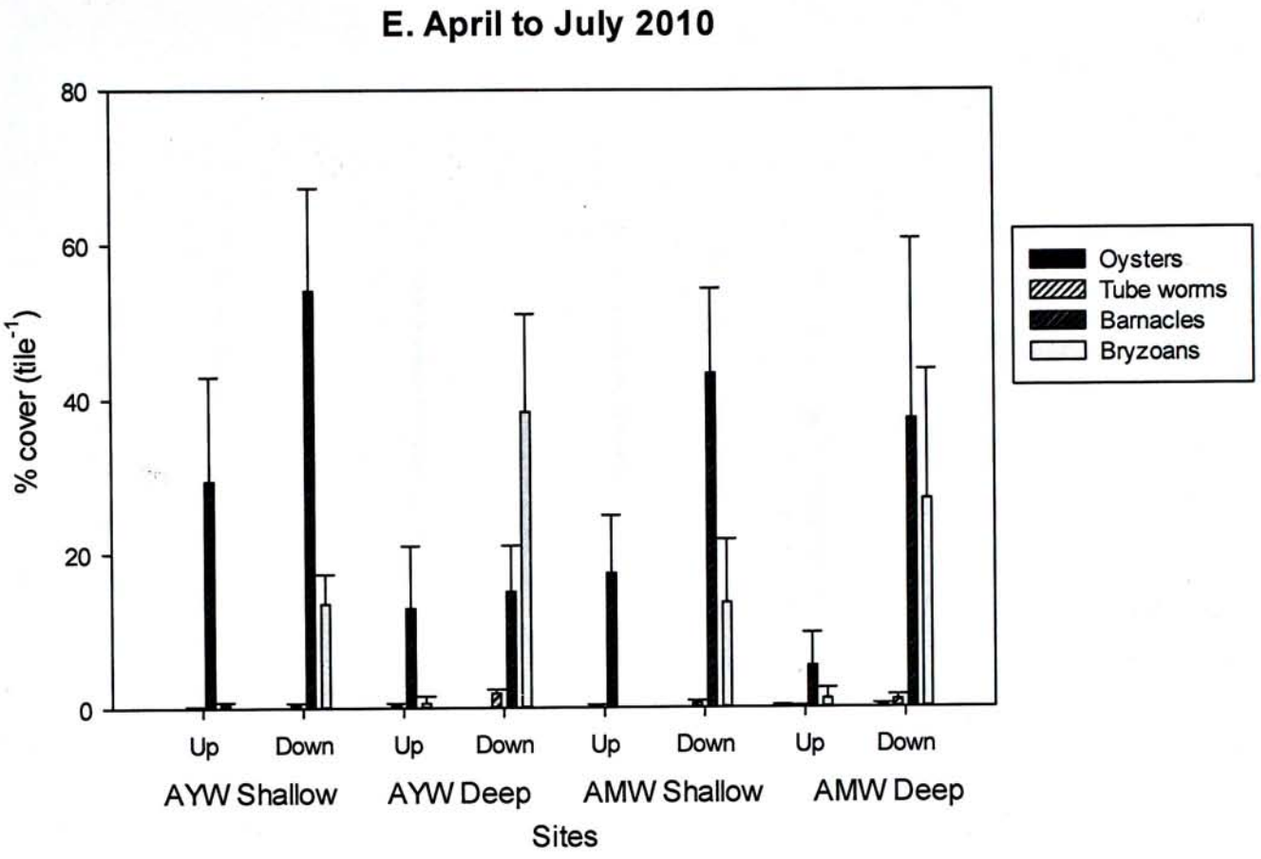


Figure 3.8 Cont'd.



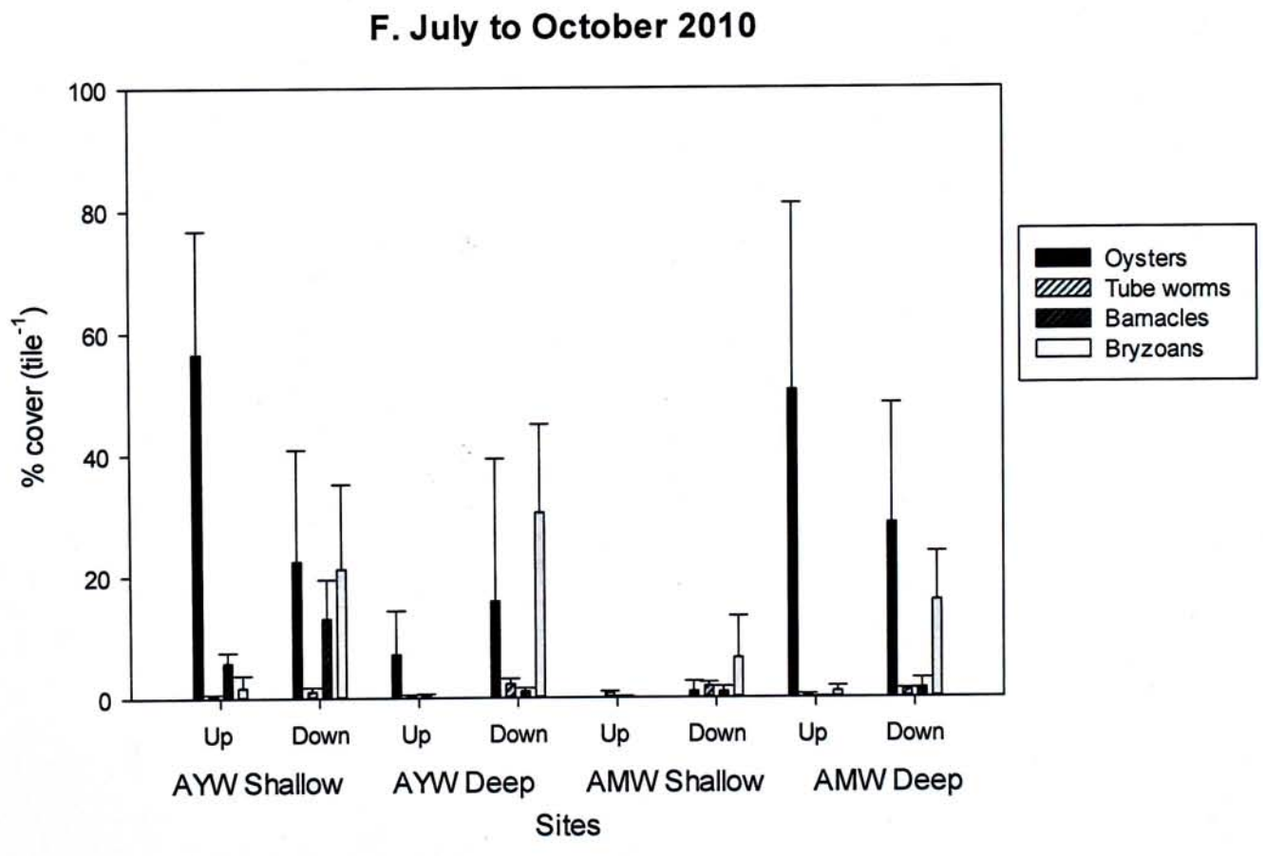
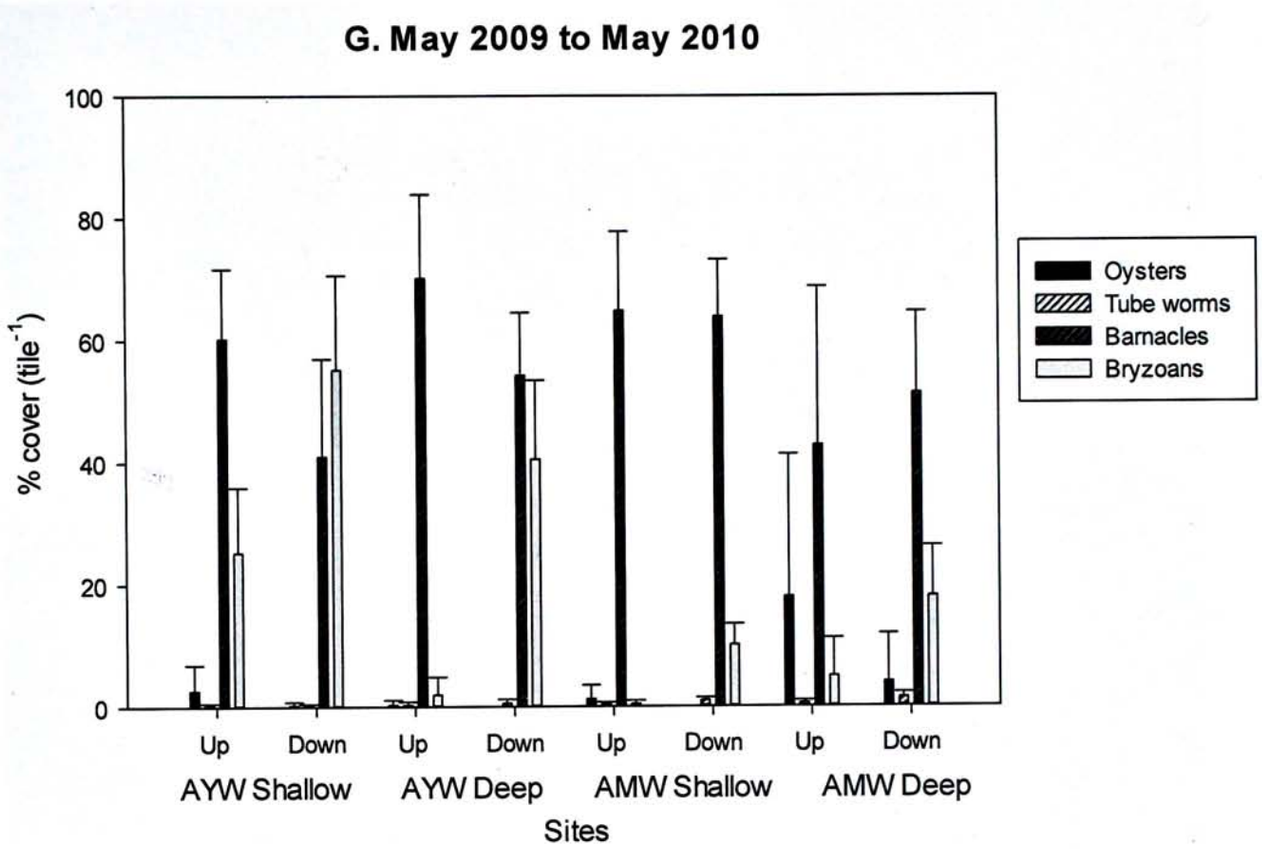


Figure 3.8 Cont'd.



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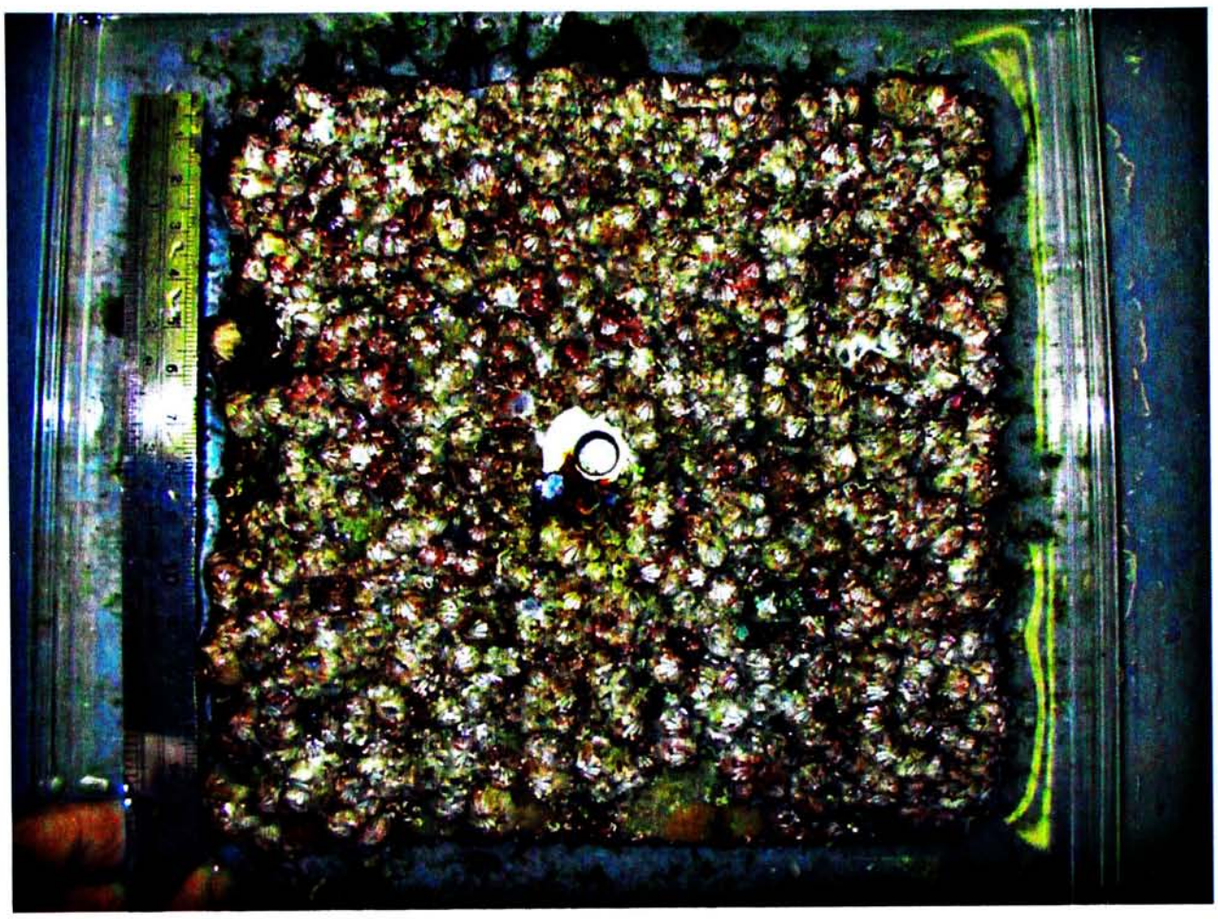


Figure 3.9 An example of the settlement tile deployed from November 2009 to January 2010 (short-term, 3 lunar month monitoring) in AYW shallow water that became almost fully covered with barnacles.

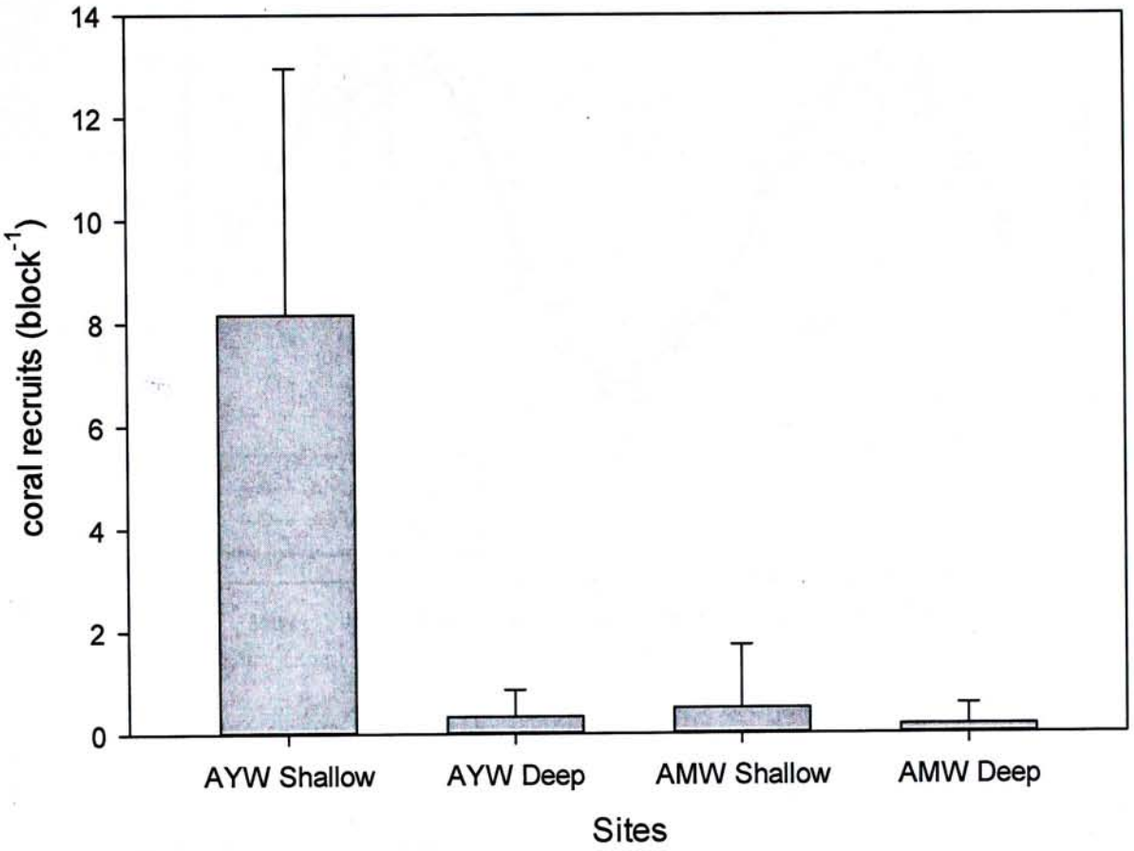


Figure 3.10 Mean (± SD) density of coral recruits on the horizontal surface of concrete blocks after 1.5 years of deployment from March 2008 to October 2009 in the four study sites in TPCMP.

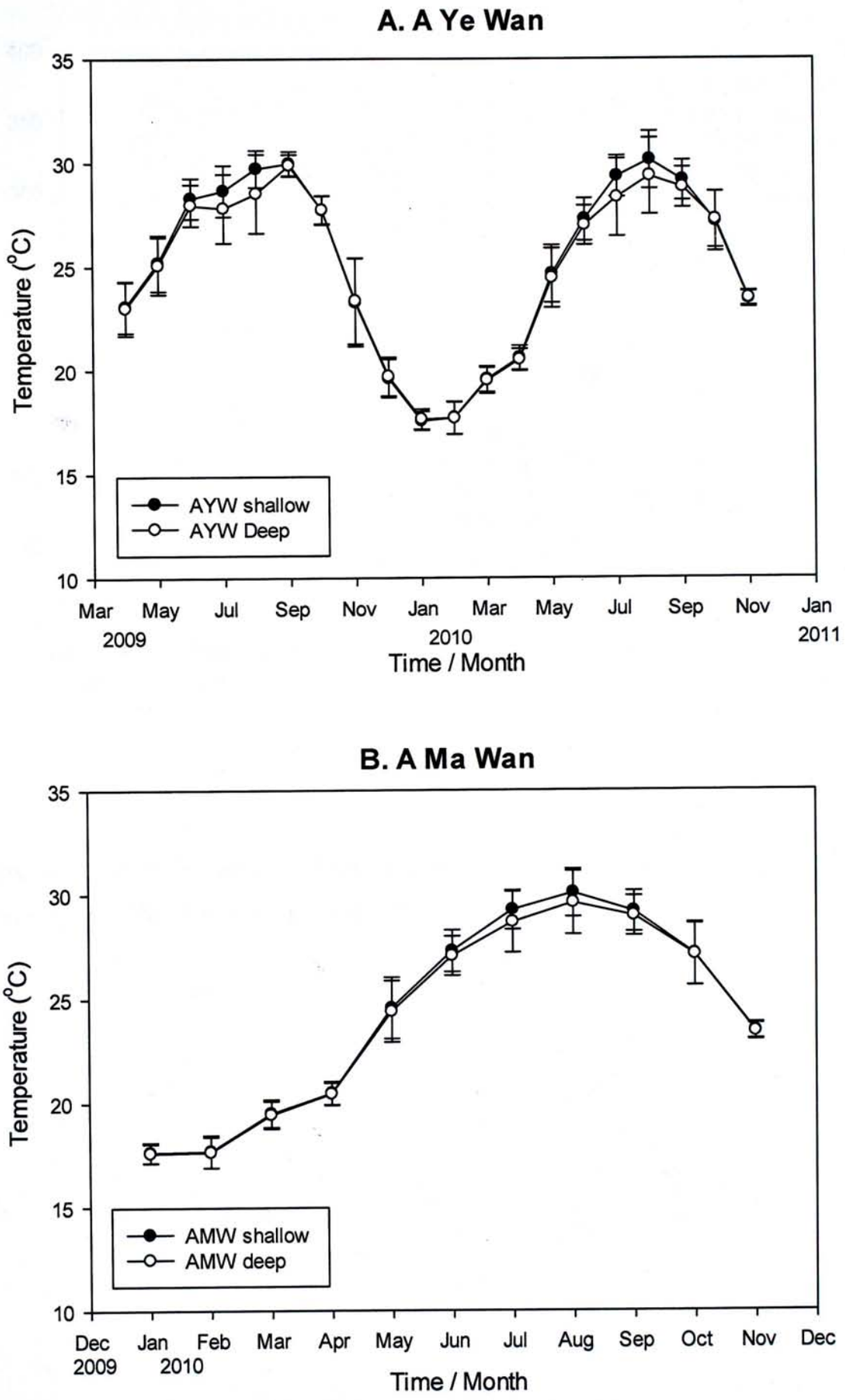


Figure 3.11 Monthly mean (± SD) seawater temperature in (**A**) A Ye Wan and (**B**) A Ma Wan from April 2009 to December 2010.

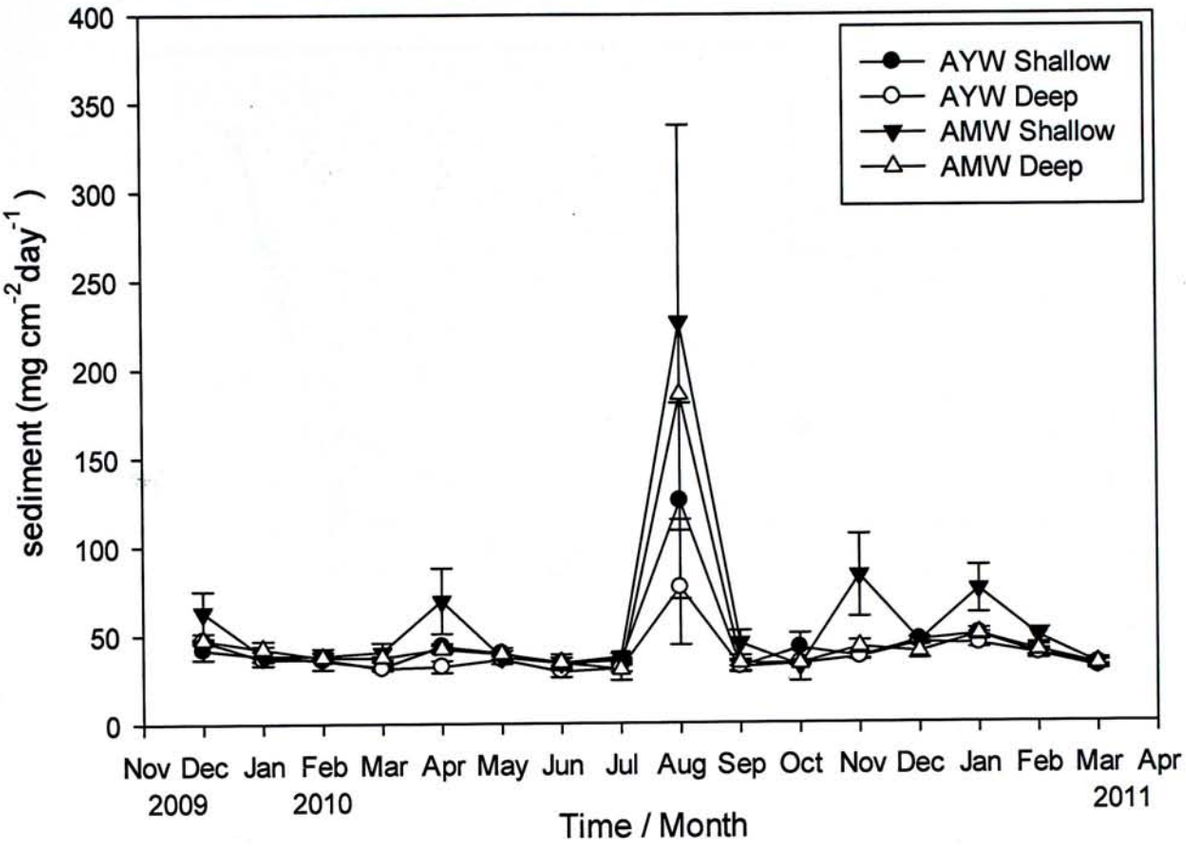
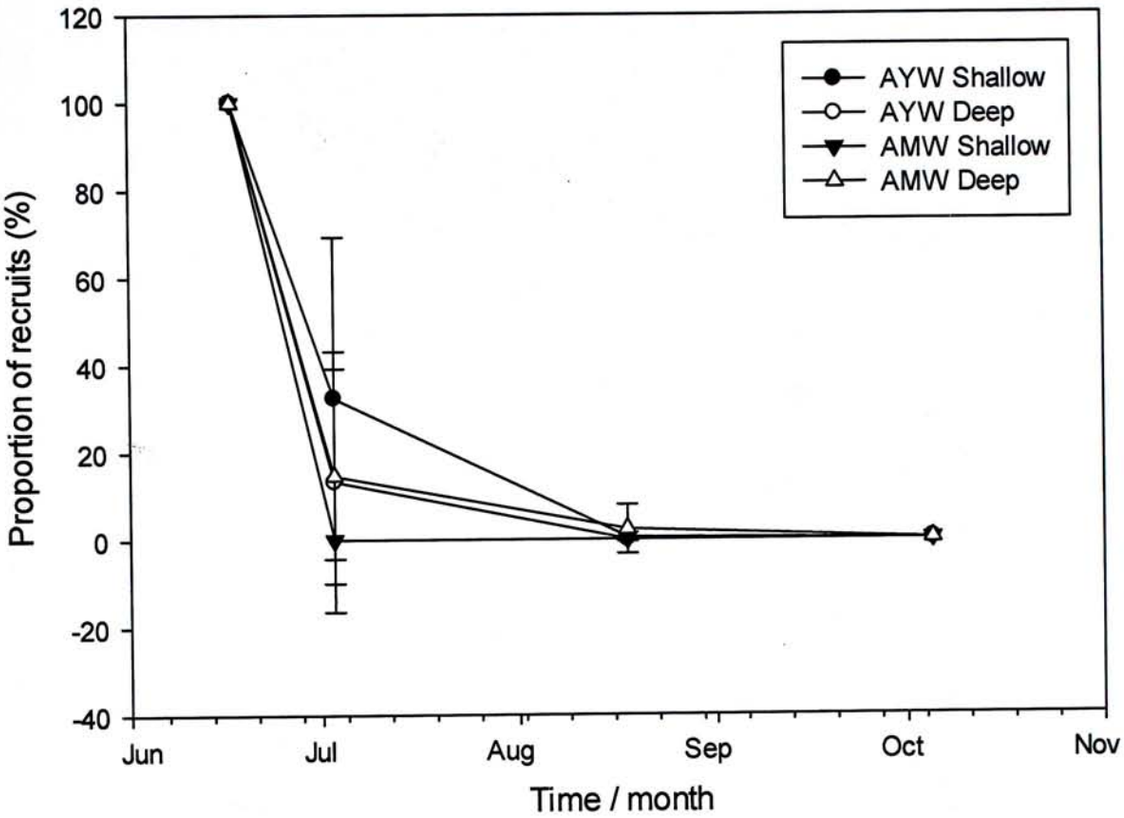
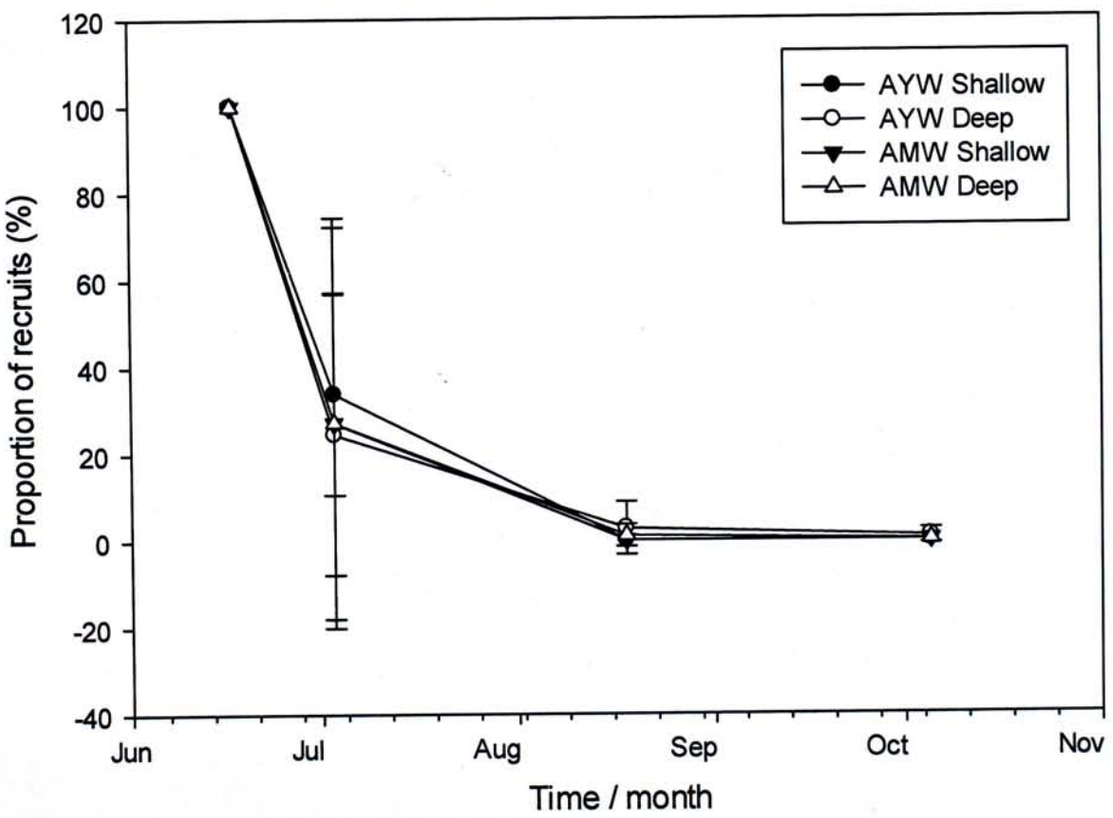


Figure 3.12 Monthly mean (± SD) sedimentation rate from November 2009 to January 2011 in the four study sites at TPCMP.

A. Post settlement survivorship in field: Face up tiles



B. Post settlement survivorship in field: Face down tiles



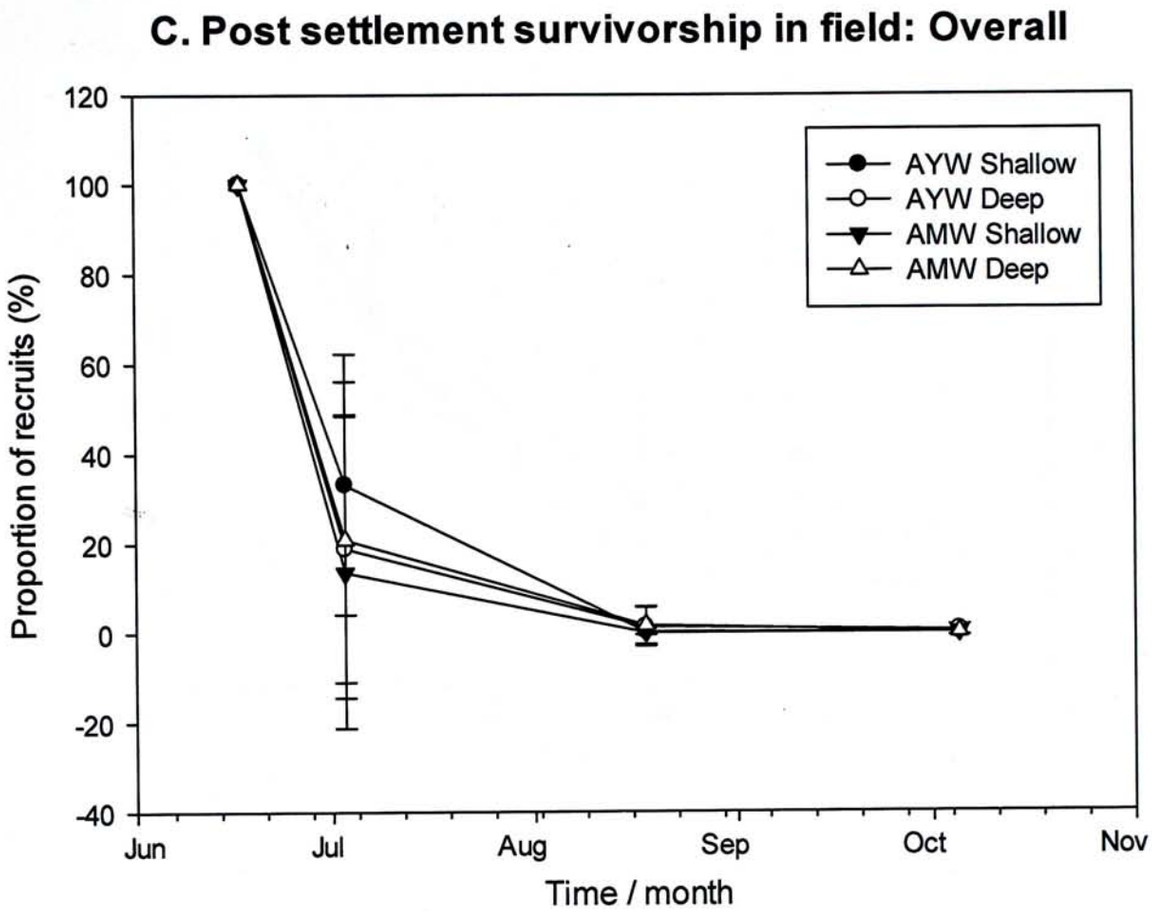
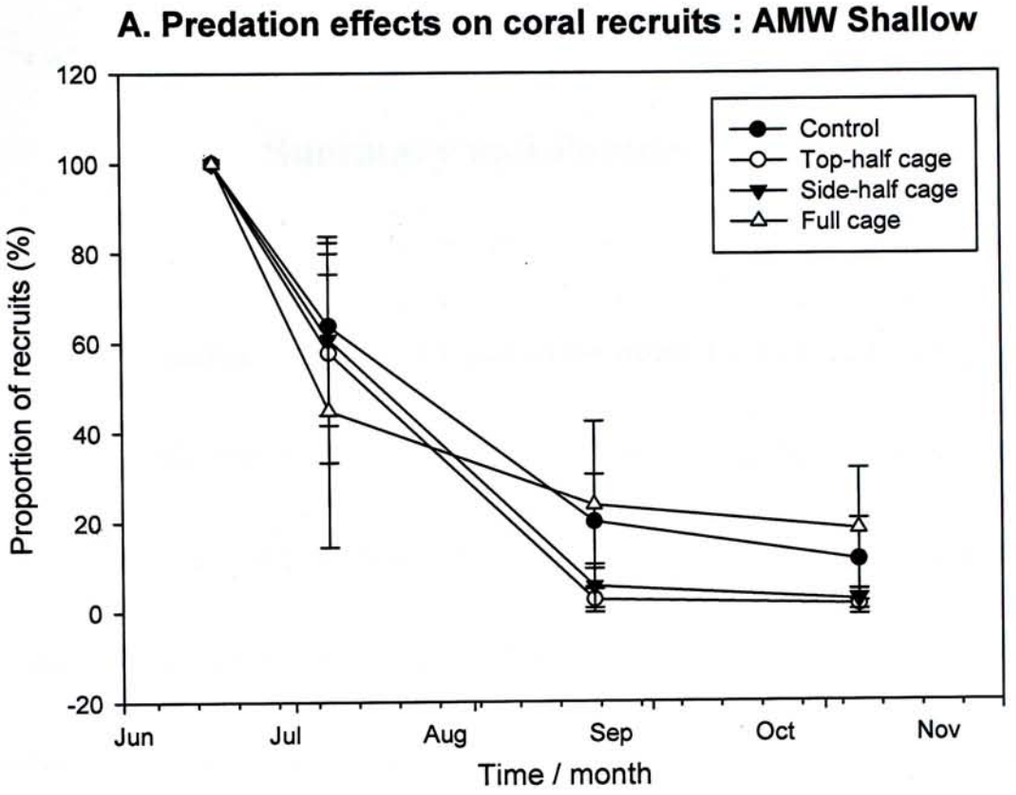


Figure 3.13 Mean (± SD) proportion (%) of coral recruits that survived on pre-seeded ceramic tiles deployed in the four study sites, TPCMP, from mid June 2010 to mid October 2010. Tiles were placed in pair on the set-up, one facing up (**A**), and one facing down (**B**). Combined data also presented in (**C**).



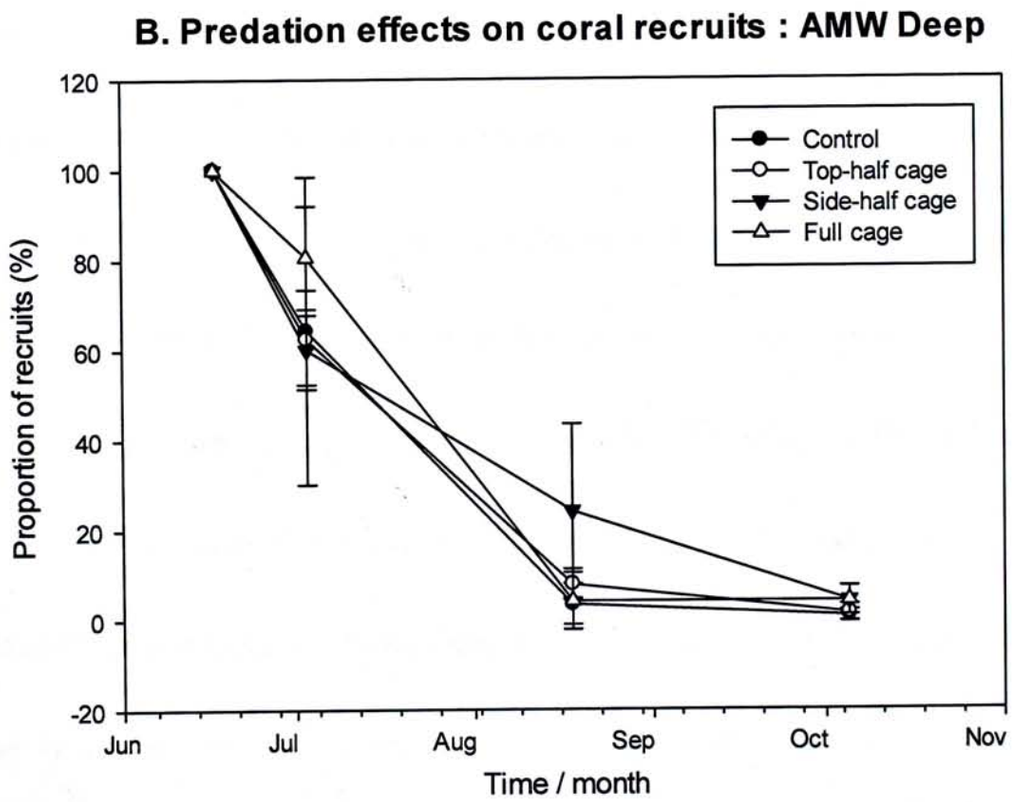


Figure 3.14 Mean (± SD) proportion (%) of coral recruits that survived on tile rods subject to different treatments and deployed in (**A**) AMW shallow and (**B**) AMW deep from mid June 2010 to mid October 2010 in TPCMP.

Chapter 4

Summary and Perspectives

Hong Kong is located at the northern part of the South China Sea, a subregion in the Indo-West Pacific that is characterized with high coral diversity. However, colder winter seawater temperature that drops to 14-16°C makes Hong Kong a marginal environment for most coral growth. Nonetheless, corals can still successfully reproduce in this inhospitable environment. Synchronous multi-specific spawning of scleractinian corals in Hong Kong was observed in May 2009, June and early July 2010, while synchronous spawning of only one scleractinian coral species was observed in late July 2010. Spawning mostly occurred from 19:30 to 22:00 hrs and involved a total of 12 species from eight genera and three families. Majority of the species observed to spawn were from the family Faviidae. Eleven species were hermaphroditic, and one species was gonochoric. This study is the first detailed documentation of spawning behavior of corals in Hong Kong. While only 12 out of a total of 84 known species in Hong Kong were observed to spawn in the present study, these 12 species nevertheless represented some of the most common and dominant species in Hong Kong.

Coral recruitment is generally recognized as one of the key processes for the maintenance of coral communities. It is a crucial process involved in facilitating reef recovery following disturbance. However, the overall data from settlement plate and concrete block experiment revealed an extremely low natural recruitment success of corals in Tung Ping Chau Marine Park (TPCMP), Hong Kong, with settlement tiles having a recruitment rate of 0.94 recruit m^{-2} and that for concrete blocks, 16.61 recruits m^{-2}, throughout the 1.5 years study period. Although recruitment rate on concrete blocks appears to be higher, this was contributed mainly (90.9%) by the recruits of *Oulastrea crispata*, a pioneering species that easily colonizes newly opened space. This species has an encrusting growth form. It thus cannot contribute to enhance the 3-dimensional topographic complexity of the coral community and is not considered to be a significant reef builder because of its small colony size (< 5 x 5 cm^2).

The overall low natural recruitment success of corals in TPCMP may be due to several factors. A lack of competent larvae retained within the sites may contribute to low settlement rate of the coral larvae. Although mass spawning of corals was observed, much of the larvae developed from the spawning events may have been drifted away from the spawning sites. TPCMP itself may not be effective in trapping

larvae from other sites, or the sources of larvae that regularly come to TPCMP may have been disturbed and thus, could no longer supply enough competent larvae that would eventually settle around the island.

Experiments were carried out to examine the other possible causes of low recruitment success. Recruits of the most common coral *Platygyra acuta* pre-seeded on ceramic tiles that were subsequently grown *in situ* showed that these coral recruits experienced very high mortality following settlement. Average mortality of 78.36% was observed within half a month of tile deployment *in situ*, and < 1% survived through the first four months of deployment. Low post-settlement survival was suggested to be a result of high sedimentation, intense competition for space with other fouling organisms and predation effects. Among the biofoulers were tube worms, oysters, barnacles and bryozoans. These biofoulers could occupy > 90% of the surface of settlement plates. Dominant biofoulers also differed between seasons. Some biofoulers, notably barnacles and bryozoans, were observed to grow over coral recruits, hence certainly contributed to their mortality over time.

Predator exclusion experiments were carried out to examine the role of coral predators like corallivorous gastropods on coral recruit mortality. The results

indicated that reducing predation effect may indirectly increase the growth of fouling organisms like oysters as they were also released from predation pressure. Thus no increase in the survival rates of the coral recruits was detected under the "predator free" condition. These results indicate a far more complex interaction among different organisms that contributed to coral recruitment failure. Some of these interactions may also be mediated by environmental factors like the extent and length of cold winter. No single factor could be pinpointed as the key that leads to low settlement rate of coral larvae or high post-settlement mortality, hence the low recruitment success. Nonetheless, the intensity of fouling does provide some clues to the extent of competition with fouling organisms for space the coral recruits must face and how this could likely contribute to high post-settlement mortality of corals.

This study provided the baseline information critical to the understanding of coral recruitment processes in Hong Kong coral communities, typified by those in TPCMP. As settlement rate of coral has also been shown to fluctuate between years and sites elsewhere outside Hong Kong (Wallace, 1985; Hughes *et al.*, 1999), a longer term monitoring of coral recruitment in a wider spatial scale is needed to provide a better picture of the coral recruitment processes in Hong Kong. The importance and significance of this understanding cannot be underestimated.

If recruitment failure is consistently repeated over years and over different sites, it means that it may take a long time for Hong Kong coral communities to recover from any natural or human induced disturbances. Or that a permanent phase shift may occur and coral communities in Hong Kong may eventually be gone forever. A case is already in point wherein part of the core area in AYW, TPCMP, destroyed by a barge during a storm in 2006, showed no sign of recovery until now (Tam and Ang, 2010). Under such circumstances, techniques to culture corals through larval rearing, either by introducing competent larvae or transplanting settled coral recruits to the denuded sites may provide an alternative to help facilitate the restoration of such disturbed coral communities.

Restoration using sexually produced propagates has the advantages of providing a higher genetic diversity, a larger number of juvenile corals, and would impose the least damage on donor coral colonies (Guest *et al.*, 2011). It is thus preferred over the use of asexually produced coral nubbins (fragments). However, larval rearing is much more labor intensive and expensive when compared with the asexual methods. Background knowledge of the reproductive patterns of the target coral species must also be known. Nonetheless, Hong Kong coral communities are usually very small. They are unlikely to be able to serve as a donor site for coral nubbins without itself

being seriously disturbed. Despites its limitations, larval rearing approach may thus be the only option available for coral restoration in Hong Kong.

The present study was successful in collecting egg-sperm bundles from the target species *Platygyra acuta*, and in achieving a high fertilization success (95.04%) of the eggs and sperms that were subsequently obtained. More than 200,000 larvae were reared, and induced to settle on artificial substrata that ended with 8,446 settled coral spats. The protocol for larval rearing and induced settlement was applied and some baseline information was generated that could be used for future application of larval rearing approach as a way to develop restoration strategy for Hong Kong coral communities. Larvae obtained from coral culture using sexual reproduction may also be used in many other studies to facilitate a new range of investigations on coral early life stages. The adaptation or susceptibility of coral early life history stages to different environmental changes can be examined to understand how these adaptations could eventually contribute to coral population and community dynamics. Other coral species in Hong Kong, e.g. the faster growing *Acropora* spp. and other dominant species, could also be studied in the near future.

Restoration using a local species is always advisable. Although massive corals like *P.*

acuta are slow growing, their being the most dominant species in Hong Kong indicates that they are well adapted to Hong Kong environment. They are therefore the logical choice as a target species for restoration works. However, the increase in the number of fouling organisms is an indication of the deteriorating quality of the seawater environment in Hong Kong that will not favor natural recruitment success of corals. With high sedimentation and intense competition for space with other fouling organisms, even the coral recruits of *Platygyra acuta* pre-seeded on ceramic tiles experienced very high mortality. While measures should be put in place to improve Hong Kong marine environment, other strategies may also be developed to enhance coral recruitment success. For example, seeded recruits on tiles could be cultured in the laboratory to a certain size before they are introduced into the field. Future works should be carried out to investigate the survival rate of different sizes of recruits *in situ*.

The increasing threats faced by Hong Kong corals and coral communities are not unique to Hong Kong but are being documented in many other places around the world. Development of future effective management strategies for the conservation and restoration of coral communities in Hong Kong should find applications in other comparable subtropical areas in southern China as well as in the Indo-west Pacific.

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